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CONCORDANCE OF SMOKING HABITS IN MONOZYGOTIC AND DIZYGOTIC TWINS

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1. PROCEDURE

THE procedure of ascertaining whether the pairs in a series of dizygotic (*i.e.* two-egg) twins differ in respect of some characteristic significantly more often than the pairs in a series of monozygotic (one-egg) twins has frequently been used in genetic studies as a means of ascertaining whether the characteristic being studied is influenced by inherited factors. Sir Ronald A. Fisher suggested that this procedure should be used as a means of ascertaining whether there was objective evidence that the differences observable among mankind, as to whether they chose to smoke and, if so, in what form, were influenced to any important extent by hereditary factors. Frhr. von Verschuer very kindly agreed to co-operate in the experiment and made available for this purpose the list of adult male like-sex twins on the Register of Twins of the Munster Institute of Human Genetics. A questionnaire (reproduced in Appendix I) was prepared by Sir Ronald Fisher and answers to the questions were sought either by mail or personal interview. Sir Ronald Fisher subsequently invited us to prepare a detailed account of this survey and we therefore examined and analysed the completed questionnaires. We have also discussed various points arising from them with Frhr. von Verschuer, with his deputy, Dr G. Koch, and with Fraulein G. Neuhauser of the Institute's staff. We are most grateful to them all for their very kind and generous assistance.

The twins on the register of the Institute of Human Genetics are classified in three " lists "—namely, the Tubingen, Berlin and Frankfurt-am-Main lists—representing the centre at which each list was originally compiled. Each " list " has had a different history which is summarised below. The lists were inevitably suspended during the war, but contact with twins was re-established where possible after Frhr. von Verschuer took up his appointment with the Institute of Human Genetics at Munster.

(i) *The Tubingen list*

Details of the history and members of the Tubingen list up to the early 1950's are given by Frhr. von Verschuer (1954) in his monograph on the subject. The list was compiled during the years 1923-27 by Frhr. von Verschuer who, as a result of his interviews with the twins, came to know them all personally. Indeed, since he first came

in contact with some of them immediately after their birth, he became godfather to a number of them.

At the outset, Frhr. von Verschuer was interested mainly in adult monozygotic twins and this accounts for the predominance of older monozygotic twins in the Tubingen list. To compile the list of twins, Frhr. von Verschuer asked those twins, whom he came across, if they knew of other twins and he also asked hospitals and schools in the Tubingen area to co-operate by asking their patients and children respectively if they happened to have a twin. Where positive replies were obtained, Frhr. von Verschuer interviewed both twins. As a result of the personal contacts which he thus established with the twins, it proved easier to re-establish the Tubingen list of twins after the war than the other lists.

In the early 1950's, when the lists were re-established, Frhr. von Verschuer succeeded in renewing a co-operating relationship with 22 monozygotic and 11 dizygotic male like-sex pairs on the Tubingen list. By the time of the enquiry conducted on behalf of Sir Ronald Fisher (Spring 1957), the numbers of these had been reduced to 19 monozygotic and 7 dizygotic pairs. The questionnaire compiled by Sir Ronald Fisher was sent to all of them. Completed questionnaires were returned by both members of 16 monozygotic and 5 dizygotic pairs; both members of 3 monozygotic and 2 dizygotic pairs did not reply. The replies received from one monozygotic pair and one dizygotic pair on the Tubingen list were not used as in each pair one of the brothers had died prior to the enquiry. The Tubingen list therefore contributed 15 monozygotic and 4 dizygotic pairs to the enquiry.

The replies of all the twins are summarised in Appendix II. In this appendix each pair is given a code number consisting of the letters " M " or " D " to indicate monozygotic or dizygotic, the letters " T ", " B " or " F " to indicate the Tubingen, Berlin or Frankfurt list, and a serial number. The two members of a pair have the same serial number and are distinguished by the addition of " a " or " b ".

(ii) *The Berlin list*

The Berlin list of twins was collected by Frhr. von Verschuer during the years 1927-42, when he was at the Kaiser-Wilhelm-Institute for Anthropology, Human Heredity and Eugenics in Berlin, through an approach to hospitals and schools as at Tubingen. The history of the Berlin list of twins has been described in a paper by Dr G. Koch (1957) of the Institute of Human Genetics at Munster. At the time Dr Koch wrote his paper (1955-56), the list included 33 monozygotic and 33 dizygotic male like-sex pairs at least one member of whom was then living in Western Berlin. Sir Ronald Fisher's request came at a time when Dr Koch was planning to visit the Berlin twins personally in order to obtain a number of other details about them and Sir Ronald's questions were therefore incorporated in the wider enquiry.

Dr Koch wrote to 21 of the monozygotic pairs and 11 of the dizygotic pairs in Berlin in order to arrange appointments with them to obtain this information at a personal interview. In selecting the twins for this purpose, Dr Koch omitted pairs in which one or both members had died or had not answered a previous communication.

Information about smoking habits was obtained from both members of all 21 monozygotic and 11 dizygotic pairs and is summarised in Appendix II. All these replies are used in this analysis.

(iii) *The Frankfurt-am-Main list*

The Frankfurt-am-Main list was compiled through the Institute of Hereditary Biology at the University of Frankfurt during the years 1935-42. For this list, the main source of information was the Frankfurt Register of Births and this was used in an attempt to obtain as complete a collection as possible of both monozygotic and dizygotic twins. Details of the history of the list are described in a monograph by Dr B. T. Duis (1956). As Dr Duis records, the male like-sex twins on the Frankfurt list in 1952 consisted of 59 monozygotic and 85 dizygotic pairs. In an enquiry conducted in 1952-53, it was found that at least one member of 13 male like-sex monozygotic pairs and 41 male like-sex dizygotic pairs was not living sufficiently close to Frankfurt to take part in that enquiry. These pairs were also excluded from the enquiry conducted on behalf of Sir Ronald Fisher, and after allowing for deaths and a few other changes, Sir Ronald Fisher's questionnaire was sent to 44 male like-sex monozygotic twins and 46 male like-sex dizygotic twins. There was less personal contact with the twins on the Frankfurt list than with those on the other two lists and while information from 4 of the monozygotic pairs was obtained by personal interview, communication with the 86 other pairs was only by correspondence. There was therefore a considerable amount of non-response. One member of 4 monozygotic and 10 dizygotic pairs and both members of 24 monozygotic and 19 dizygotic pairs did not answer. Completed answers were therefore obtained from both members of 16 monozygotic and 17 dizygotic pairs and the details are summarised in Appendix II. All are used in this enquiry. In the opinion of Frhr. von Verschuer, there are unlikely to be other reasons than personal disinclination to co-operate underlying the non-response. Dr Duis (1956) has also commented on the difficulty of obtaining response from members of the Frankfurt list.

The twins on all three lists were classified as monozygotic or dizygotic primarily on the basis of morphological characteristics. It was also possible to obtain information about the blood groups of many of the pairs, and in all cases complete similarity was found between the blood groups of the twins identified as monozygotic on the basis of morphological characteristics. Details of the blood groups, where available, are listed in Appendix II.

All twins were thus classified as monozygotic or dizygotic a long time

prior to the time at which they were asked for information about their smoking habits.

2. SMOKING HABITS OF THE TWINS

Each person in the enquiry was asked certain questions about his smoking habits at the time of the enquiry and about his past smoking history. The main particulars about his smoking habits provided by each informant are summarised in Appendix II. For a few individuals, the information was provided by someone other than the person to whom the questionnaire was addressed, and where this occurred the fact is noted in Appendix II. In all such cases, we examined whether the informant was likely to be in a position to supply accurate information and we found no reason for rejecting the information as likely to be inaccurate. Where the two twins replied in identical words, we examined the handwriting on the original replies to see if one had replied for both and we invariably found the writing to be different.

As might be expected, the informants differed widely in their smoking habits : there were relatively few classes of smoker that were not represented. For the purpose of classifying the informants according to their smoking habits, we have adopted what, we hope, are broad common-sense distinctions. The main principles of classification adopted by us are the following :—

1. The non-smokers at the time of completing the questionnaire were first distinguished from those who smoked to some extent.

2. Those who were non-smokers at the time of the enquiry were divided into those who had never smoked and those who were ex-smokers, and the latter were sub-divided according to the type of product they had formerly smoked. There were insufficient ex-smokers to justify sub-division according to the amount previously smoked or the date or age of giving up smoking.

3. Those who were smokers at the time of the enquiry were divided between regular smokers and occasional smokers. For this purpose, an occasional smoker was defined as a person who smoked less than one cigarette or one pipe a day or not more than one cigar a week (*i.e.* having a weekly consumption averaging not more than 6 cigarettes or 6 pipes or one cigar). Anyone smoking more than this was considered to be a regular smoker.

4. The regular smokers were divided into those who smoked one type of product (*i.e.* cigarettes or a pipe or cigars) exclusively or predominantly and those who smoked more than one type of product (*i.e.* mixed smokers). A smoker was considered to smoke one type of product predominantly if his consumption of other types of products was only " occasional "—*i.e.* did not average more than 6 cigarettes or 6 pipes or one cigar a week as the case might be.

5. Regular cigarette smokers were divided into smokers of packeted (*i.e.* machine-made) cigarettes, smokers who rolled their own cigarettes (*i.e.* hand-rolled cigarettes) or both.

6. Cigar smokers were divided into cigar or stumpen * smokers respectively. Stumpen smokers were defined as those who specifically mentioned stumpen in their replies although it was appreciated that, as questions had not been asked about stumpen as such, some smokers of stumpen might have replied in terms of the generic heading of cigars. The consumption of cigarillos, not uncommon in Germany, was not specifically asked about nor specifically mentioned in replies.

* Stumpen are cigars of slightly shorter length generally having a rectangular cross-section.

7. Mixed smokers were divided into appropriate groups.

8. Smokers of packeted cigarettes were sub-divided into three broad consumption levels. There were insufficient smokers of the other types of product to justify sub-division by level of consumption, and in particular there was the possibility that some of the quantities mentioned by cigar smokers really referred to stumpen or cigarillos.

9. Six informants supplied insufficient information to enable them to be completely classified.

If the 168 men whose replies have been used in this enquiry, are classified according to their smoking habits, using the foregoing principles, the following figures are obtained :—

TABLE 1

Smoking habits of twins in the enquiry

Classification code no.	Classification	Monozygotic individuals	Dizygotic individuals
	Non-smokers		
1	Non-smokers who had never smoked	21	17
	Non-smokers who are ex-smokers—		
2	(*a*) Non-smokers who formerly smoked cigarettes exclusively or predominantly	3	3
3	(*b*) Non-smokers who were ex-smokers of cigarettes and cigars, having formerly smoked both more than occasionally	1	...
4	(*c*) Non-smokers who were ex-smokers but did not specify the type of product previously smoked	2	3
	Occasional smokers—		
5	(*a*) of cigarettes	4	1
6	(*b*) of cigars	...	3
7	(*c*) of a pipe	...	1
8	(*d*) who did not specify the product usually smoked	...	1
	Regular cigarette smokers—		
	Packeted cigarette smokers—		
9	(*a*) very light smokers (less than 5 cigarettes a day)	7	3
10	(*b*) moderate cigarette smokers (5-24 cigarettes a day)	48	19
11	(*c*) heavy cigarette smokers (25 or more cigarettes a day)	1	...
12	Cigarette smokers who roll their own cigarettes	1	...
13	Smokers of both packeted and hand-rolled cigarettes	...	1
	Regular stumpen and cigar smokers—		
14	(*a*) Stumpen smokers	2	1
15	(*b*) Cigar smokers	7	3
16	*Regular pipe smokers*	1	2
	Regular mixed smokers—		
17	(*a*) Cigarettes and cigar smokers	3	...
18	(*b*) Cigarettes and pipe smokers	1	4
19	(*c*) Cigar or stumpen and pipe smokers	2	2
	Total	104	64

3. THE CONCORDANCE OF SMOKING HABITS IN MONOZYGOTIC AND DIZYGOTIC TWINS

In classifying the twins according as the smoking habits of the members of a pair of twins are concordant or discordant, a number of questions arise when seeking to decide what constitutes similarity of habit. For example, should a man who has ceased to smoke cigarettes be considered as similar in habit to one who still smokes cigarettes or to a non-smoker or is he in a category by himself and dissimilar to both? Is an occasional smoker of cigarettes basically similar in habit to a non-smoker or to a regular but light cigarette smoker or does he differ from both? Corresponding difficulties also arise in deciding whether a smoker who smokes one type of product exclusively or predominantly (*i.e.* a second type of product only occasionally) is similar or dissimilar to a smoker who smokes both types of product more than occasionally.

There is no simple solution to such problems. All that can be done is to state clearly the bases of classification that have been adopted and to consider all reasonable alternative bases for classification. We have therefore considered a number of alternatives and since the details given in Appendix II are sufficient to enable any alternative classification to be considered, we set out below only the main alternative bases of classification that we have examined.

(i) *Concordance in respect of smoking in any form*

The first basis of comparison between the monozygotic and dizygotic twins was whether or not the informants were smokers. The theoretically clear-cut distinction between smokers and non-smokers was blurred by two marginal groups—those who were occasional smokers and those who formerly smoked but no longer were regular smokers at the time of the enquiry (*i.e.* the ex-smokers). Consequently, the informants were analysed on three different bases.

(a) *Analysis into smokers and non-smokers.* In this analysis, occasional smokers were treated as being smokers and ex-smokers as being non-smokers. Consequently, if the members of a pair of twins were *either* both present non-smokers (whether or not they were ex-smokers) *or* both present regular or occasional smokers, the pair were classified as " like " in their smoking habits. Otherwise, they were classified as " unlike ". This analysis gave the following results :

TABLE 2

Concordance in respect of smoking—Analysis 1

Smoking habits	Number of pairs	
	Monozygotic	Dizygotic
Like	43	19
Unlike	9	13
Total . . .	52	32

(b) *Analysis into regular smokers and others.* In this analysis, the ex-smokers and occasional smokers were treated as being non-smokers and smokers were taken to comprise only those who were regular smokers at the time of the enquiry. Consequently, if the members of a pair of twins were *either* both non-smokers, ex-smokers or occasional smokers *or* both present regular smokers, the pair were classified as " like " in their smoking habits. Otherwise, they were classified as " unlike ". This analysis gave the following table :

TABLE 3

Concordance in respect of smoking—Analysis 2

Smoking habits	Number of pairs	
	Monozygotic	Dizygotic
Like	43	17
Unlike	9	15
Total . . .	52	32

(c) *Analysis into " smoked sometime " and " never smoked ".* In this analysis, occasional smokers and ex-smokers were both treated as smokers. Consequently, if the members of a pair of twins were both *either* present or past regular or occasional smokers *or* had both never smoked, the pair were classified as " like " in their smoking habits. Otherwise, they were classified as " unlike ". The analysis on this basis gave the following results :

TABLE 4

Concordance in respect of smoking—Analysis 3

Smoking habits	Number of pairs	
	Monozygotic	Dizygotic
Like	47	25
Unlike	5	7
Total . . .	52	32

(ii) *Analysis by type of product smoked*

The next step was to classify the twins according to the type of products smoked. As before, a number of alternative analyses had to be examined. The following analyses are in terms of the products smoked by the informants at the time they replied to the questionnaire. If ex-smokers were classified according to the type of products

they last smoked, we would be classifying ex-smokers according to the products they smoked, say, 2, 5 or 10 years ago and current smokers according to the products which they smoked at the time of the enquiry and which might have differed from those smoked in past years. We do not know the precise types of product and quantities smoked by the twin of an ex-smoker at the time the latter gave up smoking.

(a) *Analysis by types of products and amounts smoked.* In table 1, in which the informants were classified according to their smoking habits, there were altogether 19 different categories. Two of these categories (namely, categories 4 and 8) were in the nature of residual categories into which informants were classified when they had provided insufficient information about types of products smoked or formerly smoked. This therefore left 17 detailed categories into which the informants were analysed when they provided full information about their smoking habits. Consequently, the two members of a pair of twins were considered as " like " if they both fell into the same one of these 17 groups. Otherwise they were classified as " unlike ". This analysis yielded the following results :

TABLE 5

Concordance in respect of products smoked—Analysis 1

Smoking habits	Number of pairs	
	Monozygotic	Dizygotic
Like	26	7
Unlike	24	21
Total . . .	50	28

(b) *Analysis by types of products and amounts smoked (reduced number of categories).* Since it might be argued that the classification of informants into 17 categories for the purpose of the foregoing analysis overstated the distinctions that really existed between smokers, an analysis was therefore made in which the number of different categories was reduced. Thus, non-smokers, ex-smokers and occasional smokers were treated as being in effect non-smokers ; smokers of packeted and hand-rolled cigarettes were treated as both being cigarette smokers and the distinction between the different levels of cigarette smokers was dropped. Consequently, the two members of a pair of twins were treated as " like " if they both fell into any one of the following 7 categories and " unlike " if they fell into different categories :

1. Non-smokers, ex-smokers and occasional smokers.
2. Cigarette smokers.
3. Stumpen or cigar smokers.
4. Pipe smokers.

5. Cigarette/cigar smokers.
6. Cigarette/pipe smokers.
7. Cigar or stumpen/pipe smokers.

The classification of each pair of twins on this basis gave the following results :

TABLE 6

Concordance in respect of products smoked—Analysis 2

Smoking habits	Number of pairs	
	Monozygotic	Dizygotic
Like	33	11
Unlike	19	21
Total . . .	52	32

(c) *Analysis by regularly-smoked products.* In the preceding analyses, those who smoked more than one type of product, except where the second product was smoked only occasionally (as already defined), were treated as being in a separate category from those who smoked only one of the products concerned—*i.e.* those who smoked both cigarettes and cigars were considered as falling into a different category from those who smoked only cigarettes. It might be argued, however, that the two members of a pair of twins who smoked the same product either by itself or in combination with some other product should be regarded as " like " in their smoking habits. In other words, if one twin smoked cigars *and* cigarettes and his brother smoked either cigarettes *or* cigars, the pair should be regarded as " like " in their smoking habits since they had in common a product which was either their main form of consumption of tobacco or at least a considerable proportion of their total consumption of tobacco. For this analysis, packeted and hand-rolled cigarettes were both regarded as cigarettes, and stumpen and cigars were both regarded as cigars ; occasional smokers and ex-smokers were regarded as non-smokers. The twins were therefore re-classified on this basis and this gave the following results :

TABLE 7

Concordance in respect of products smoked—Analysis 3

Smoking habits	Number of pairs	
	Monozygotic	Dizygotic
Like	38	15
Unlike	14	17
Total . . .	52	32

(d) *Analysis on the basis of direct comparison between twins.* All the foregoing analyses of twins into pairs who were " like " or " unlike " in their smoking habits have in effect involved a two-stage classification procedure. That is, each informant was first classified into one of a number of categories according to his current and past smoking habits and then each informant was compared with his twin to see if the two had been put into smoking categories which, for the purpose of the particular analysis, were accepted as being the same. This procedure had the advantage that all the criteria required for classification of the twins were explicitly stated and that the exercise of personal judgment was almost wholly eliminated. The procedure was completely reproducible and every person repeating the classification should obtain the same result. On the other hand, this reproducibility and relative simplicity had two disadvantages. Firstly, it did not allow for cases in which concordance of smoking habit was doubtful. For each pair of twins, the decision was either " like " or " unlike " but there were, on any set of definitions, a number of pairs whose differences in smoking habits were marginal. Secondly, the procedure opened the door to certain " forced distinctions ". Thus, a person smoking 6 cigarettes a week would be classified as an " occasional " smoker, while one smoking 8 cigarettes a week would be classified as a " regular " cigarette smoker. This example did not in fact occur—in the nearest actual case, one man smoked 5 cigarettes a week and his twin 21-35 cigarettes a week, but the possibility of " forced distinctions " remains inherent in the procedure. This type of forced distinction can, however, be met by introducing a larger degree of personal judgment in assessing the concordance of each pair's smoking habits. Further, the procedure adopted had the disadvantage that certain subtle differences were inevitably neglected. For example, in the case of ex-smokers no account has been taken of the extent to which external factors (in the form of major illness or medical advice) forced an otherwise willing smoker to give up smoking. In the case of occasional cigarette smokers, no account has been taken of the way in which they described themselves. Their descriptions of themselves were, in fact, about equally divided between " smoker " and " non-smoker ". In the case of those who occasionally smoked a second type of product in addition to regular smoking of some other product, no account has been taken of the fact that the smoking of a small number of cigarettes by one who was normally a cigar or pipe smoker might be a very minor matter, which could be regarded as little more than a social gesture, whereas even the occasional smoking of a pipe, as a second form in which tobacco was taken, would be of much more significance since it at least involved the carrying of a pipe for the purpose. These distinctions can, of course, be introduced by specifying much more detailed bases of classification.

Sir Ronald Fisher has personally made an assessment of the concordance of the smoking habits of the members of each pair of

twins, taking such points into consideration and using the categories of " like ", " doubtful " and " unlike ", with the following results :

TABLE 8*

Concordance in respect of products smoked—Analysis 4

Smoking habits	Number of pairs	
	Monozygotic	Dizygotic
Like	34	11
Doubtful . . .	6	4
Unlike	12	17
Total . . .	52	32

* Information about two additional pairs of twins has become available since Sir Ronald Fisher (1958) made a preliminary analysis.

4. DISCUSSION

The three lists of twins are obviously not homogeneous. The lists were compiled at different times, in different ways, for different purposes, with a different degree of closeness of relationship to the compiler and with different histories of loss and wastage. In the present enquiry, the proportion of non-response was different in the different lists. In particular, the non-response was greatest in the Frankfurt list, although in this list it occurred in the two types of twins in practically equal proportions. Nevertheless, we see no reason why any of these differences should have produced bias or unrepresentativeness in the twins as far as smoking habits were concerned. Indeed, from the point of view of smoking habits, many of the twins were obtained on a " prospective " basis—*i.e.* they were recruited many years before they started to smoke.

The analysed questionnaires of the Tubingen list covered 15 monozygotic and 4 dizygotic male like-sex twins, the Berlin list 21 monozygotic and 11 dizygotic pairs and the Frankfurt list 16 monozygotic and 17 dizygotic pairs. The age distribution of the 84 pairs is shown in table 9 on page 428.

As will be seen, the monozygotic twins in the Tubingen and Berlin lists are older on the average than the dizygotic twins. The two sets of Frankfurt twins are more closely matched in age, doubtless as a result of the different method of recruitment already described. If, as may well happen, the passage of time encourages divergences in the smoking habits of twins, the difference in average age between the Tubingen and Berlin monozygotic and dizygotic twins would produce differences between the smoking habits of the monozygotic twins to a greater extent than between those of the dizygotic twins.

In this event, the preceding analyses would have understated the greater degree of concordance that exists in the smoking habits of monozygotic twins as compared with dizygotic twins.

TABLE 9

Age distribution of twins used in main analyses

Age	Monozygotic twins				Dizygotic twins			
	Tubingen	Berlin	Frankfurt	Total	Tubingen	Berlin	Frankfurt	Total
20-24	...	1	3	4	...	4	5	9
25-	...	7	5	12	...	4	4	8
30-	...	2	1	3	1	1	2	4
35-	2	3	1	6	2	1	2	5
40-	5	2	3	10	...	1	...	1
45-	5	1	...	6	...	...	2	2
50-	1	...	2	3	1	...	1	2
55-	1	2	...	3	...	...	...	...
60-	...	1	...	1	...	...	...	...
65-	...	1	1	2	...	...	...	...
70+	1	1	...	2	...	...	1	1
Total	15	21	16	52	4	11	17	32

On the other hand, it might be argued that the two members of the monozygotic pairs may be found more frequently to be living in close proximity to each other than dizygotic twins and that it is this proximity, rather than any genetic factor, which has produced the greater concordance that exists in the smoking habits of monozygotic twins. We therefore analysed the degree of proximity of residence of the twins, distinguishing the three lists. The results were as follows :

TABLE 10

Proximity of residence

List	Monozygotic twins			Dizygotic twins			Total
	Same address same town	Different address same town	Different town	Same address same town	Different address same town	Different town	
Frankfurt .	7	6	3	2	5	4	33
Tubingen .	3	4	2	1	1	2	19
Berlin . .	8	8	5	7	2	2	32
Total .	18	18	16	16	8	8	84

Thus, in Frankfurt, the monozygotic twins and dizygotic twins were almost equally distributed between the three different categories of proximity ; elsewhere, the dizygotic twins were proportionately

found more frequently at the same address than the monozygotic twins. If proximity of residence encourages similarity of smoking habits, then greater concordance is to be expected between the dizygotic than the monozygotic pairs in the Tubingen and Berlin lists. There is, therefore, no evidence to suggest that the greater concordance of smoking habits in monozygotic pairs than in dizygotic pairs was due to more frequent proximity of residence.

In fact, if the monozygotic and dizygotic pairs are combined and analysed for concordance of smoking habits in terms of table 2 (*i.e.* according as the pairs of twins are " like " or " unlike " in respect of being smokers or non-smokers), the following relationship between concordance of habit and proximity of residence is obtained :

TABLE 11

Proximity of residence and concordance of smoking habit by twin pairs—I

Smoking habits (per analysis used in table 2)	Same address same town	Different address same town	Different town
Like	24	19	19
Unlike	10	7	5
Total	34	26	24

Thus proximity of residence of the members of a pair of twins does not appear to exercise any marked influence in leading them both to be smokers or both to be non-smokers. On the other hand, if both members of the pair do in fact smoke, there is more frequent concordance in respect of the types of product smoked between the members of pairs resident at the same address in the same town than between the members of pairs resident in different towns. The following table compares proximity of residence and concordance of smoking habit in terms of the definition of concordance used in table 6 above :

TABLE 12

Proximity of residence and concordance of smoking habit by twin pairs—II

Smoking habits (per analysis used in table 6)	Same address same town	Different address same town	Different town
Like	20	14	10
Unlike	14	12	14
Total	34	26	24

The pairs living at the same address are, as might be expected, very much younger on average than the pairs living apart.

When the concordance or discordance of smoking habits in monozygotic and dizygotic pairs is analysed according to the three lists, it is found on the basis of the analysis used in table 6, that the Frankfurt list is markedly different from the other two lists as the following table shows :

TABLE 13

Smoking habits

	Monozygotic pairs			Dizygotic pairs		
	Alike	Unlike	Total	Alike	Unlike	Total
Tubingen . .	7	8	15	1	3	4
Berlin . . .	13	8	21	6	5	11
T and B . .	20	16	36	7	8	15
Frankfurt . .	13	3	16	4	13	17
	33	19	52	11	21	32

All the evidence, however, points to the Frankfurt list as being equally or more representative than the other two. Thus,

(1) As already stated, the monozygotic and dizygotic twins are more closely matched in age in the Frankfurt list than in the other lists, and the higher average age of the monozygotic twins, as compared with dizygotic twins, in the Tubingen and Berlin lists may tend to understate the degree of concordance of smoking habits in the monozygotic twins to the concordance in dizygotic twins.

(2) The method of recruitment adopted in collecting the Frankfurt list would be more likely to ensure collection of a totally unbiased sample of the two types of twins.

(3) Frhr. von Verschuer (1939) has expressed the view " that only every fourth pair of twins is monozygotic ". Since all monozygotic twins are of the same sex, every eighth pair of twins would consist of monozygotic male twins and, correspondingly, 3 of every 16 pairs of twins would be male dizygotic twins. The expected ratio of male monozygotic twins to male dizygotic twins in a random sample of the population would therefore be 2 to 3. The number of monozygotic and dizygotic pairs of male like-sex twins in the Frankfurt list in 1952 was 59 and 85. On a 2 to 3 ratio the expected numbers in a total of 144 male like-sex twin pairs would be 58 monozygotic pairs and 86 dizygotic pairs respectively. The ratio of the numbers of twins of the two types in the Frankfurt list in 1952 was therefore extremely close to what might have been expected on a random sample

of the population. We see no reason why any of the wastage or non-response that subsequently occurred would be likely to have introduced a bias favouring concordance in the smoking habits of monozygotic twins.

(4) We have compared the numbers of smokers found among the dizygotic twins in each of the lists—a " smoker " for this purpose being a person who defined himself to be a smoker —with the number that would have been expected in an equal number of randomly selected and unrelated men of the same ages, in view of the percentage of smokers found in Western Germany as a whole in 1957. The figures are as follows :

TABLE 14

Expected and actual smokers among dizygotic twins

List	Dizygotic twins	
	Expected number of smokers	Observed number of smokers
Frankfurt . . .	25	20
Tubingen and Berlin .	22	17

While it is, of course, the concordance in the smoking habits of the dizygotic pairs that has been used throughout as the control by which the characteristics of the monozygotic pairs have been judged, the figures in table 14 show that, in incidence of smoking, the dizygotic pairs in the Frankfurt list and in the other two lists combined are representative of the public as a whole to an equal degree.

We can therefore see no reason for doubting the reality of the definitely more frequent occurrence of concordance of smoking habits in monozygotic than in dizygotic twins. Although the data were not designed for the application of chi-square tests, the formal probabilities of getting as great or greater departures from those expected on the null hypothesis of equal concordance supply satisfactory indications that the disparity of proportions observable between the two classes of twins is not reasonably ascribable to chance.

Frhr. von Verschuer and his colleagues had carried out a serological analysis of the blood of many of these twins and the available information has been recorded in Appendix II. As a first step in analysing the nature of any genetic factor that may be thought to influence smoking habits, we have analysed the blood groups of the present smokers (including occasional smokers), ex-smokers and non-smokers respectively to see if any marked differences were found in their blood groups. The results are set out in table 15 overleaf.

5. SUMMARY

1. An enquiry was carried out to ascertain whether there was objective evidence that the differences in smoking habits among mankind were influenced to any important extent by hereditary factors. For this purpose, information about the smoking habits of adult male like-sex twins on the Register of Twins of the Munster Institute of Human Genetics was obtained by mail or personal interview. The analyses were based on replies from 52 monozygotic and 32 dizygotic pairs of twins.

2. The twins were classified according to whether the smoking habits of the members of a pair were concordant or discordant.

TABLE 15

Blood groups in relation to smoking or non-smoking

Blood groups	Smoker	Ex-smoker	Non-smoker	Total
A	23	3	5	31
A_1	2	...	2	4
A_2	2	...	2	4
$A_{2(1)}$	1	...	...	1
A_1B	2	...	...	2
AB	4	...	...	4
B	5	1	...	6
O	17	1	9	28
Total	56	6	18	80
M	14	1	1	16
N	14	2	5	21
MN	26	3	12	41
Total	54	6	18	78
Rh+	9	1	1	11
rh−	3	...	4	7
Total	12	1	5	18

The first basis of comparison was whether or not the informants were smokers. Several different definitions of a smoker were used in turn and on all these definitions, concordance of smoking habits was found more frequently in monozygotic than in dizygotic twins.

3. The twins were also classified according to the types of product smoked. As before, a number of alternative bases of classification were used and again it was found that the smoking habits of monozygotic twins were more concordant than those of dizygotic twins.

4. A number of factors which might be thought to have affected the comparability or representativeness of the two sets of twins was discussed. As a result, it was concluded that there would seem to be no reason for doubting the reality of the more frequent occurrence of concordance of smoking habits in monozygotic than in dizygotic twins.

5. Information about the blood groups of many of the twins in the enquiry was available and was analysed according to smoking habits.

6. REFERENCES

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APPENDIX I

QUESTIONNAIRE

1. Name and Profession.
2. Age.
3. Place and date of birth.
4. Domicile.
5. Are you a smoker now or a non-smoker?
6. Do you smoke predominantly cigarettes, cigars or a pipe?
7. Do you take snuff?
8. Do you chew tobacco?
9. What is your daily (weekly) consumption of cigarettes, cigars, tobacco, chewing tobacco and snuff?
10. When did you smoke for the first time?
11. Have you smoked continuously since then?
12. When and why did you stop smoking?
13. Did you smoke less or more after marrying?
14. Have you smoked less or more during an illness, during the war, or for economic reasons?

APPENDIX II

Abbreviations used

Number	*Proximity of residence*	*Classification of smoking habits*
M = Monozygotic	A = Same town, same address	As per Table 1
D = Dizygotic	B = Same town, different address	
T = Tubingen list	C = Different town	
B = Berlin list		
F = Frankfurt-am-Main list		

NOTE.—Details of changes in consumption due to wartime supply difficulties have been omitted from " Past Smoking History ".

Monozygotic twins

Number	Age at 1.1.58	Proximity of Residence	Smoking habits at time of enquiry	Past smoking history	Classification of smoking habits	Blood groups and factors
M-T-1*a*	40	A	5-6 cigarettes a day	Began at 17 or 18, smoked less after marriage	10	...
M-T-1*b*	40	A	"Rather heavier smoker" (of cigarettes) than his brother (*Note:* M-T-1*b* asked M-T-1*a* to reply on his behalf)	...	10	...
M-T-2*a*	78	B	4-5 cigars a week	Began at 16. Gives up smoking during illness	15	...
M-T-2*b*	78	B	1-2 cigars a day but this is slightly less than usual on account of illness	Began at 18	15	...
M-T-3*a*	44	A	Some 15 filter tipped cigarettes a day "according to my mood"	Began at 18	10	...
M-T-3*b*	44	A	"Non-smoker for 3½ years"	Formerly smoked cigarettes; began at 18; gave up 3½ years ago after illness "on doctor's advice and at my own wish"	2	...
M-T-4*a*	53	B	50 cigars and 1 packet of tobacco a week	Began at 18. Smoked mainly cigarettes before marriage	19	...
M-T-4*b*	53	B	5 cigars a day	Began at 18. Smoked less after marriage	15	...
M-T-5*a*	48	C	Very heavy cigarette and cigar smoker	Began at 20. Smoked more after marriage	17	...
M-T-5*b*	48	C	8-10 stumpen a day (in a pipe-type holder)	Began at 28. Gives up when ill	14	...
M-T-6*a*	58	C	5-8 cigars a day, more on Sundays and certain festive occasions	Began at 18. Smoked cigarettes and pipe also during war. Usually gives up when ill. Quantity smoked varies as funds permit	15	...
M-T-6*b*	58	C	12 cigarettes and about 10 cigars a week. Describes himself as a cigar smoker	Began at 18. Gives up when ill	17	...

Monozygotic twins—continued

Number	Age at 1.1.58	Proximity of residence	Smoking habits at time of enquiry	Past smoking history	Classification of smoking habits	Blood groups and factors
M-T-7*a*	49	C	About 5 cigarettes a week mainly when in company and therefore intermittently. Describes himself as a "smoker"	Began at 17. Gave up periodically on account of asthma. Smoked more when aged 20 to 30	5	...
M-T-7*b*	49	C	3-5 cigarettes a day, increasing up to 10 in company	Began at 22. Gives up during respiratory illness	9	...
M-T-8*a*	48	B	Non-smoker	...	1	...
M-T-8*b*	48	B	An occasional cigarette. Describes himself as a "smoker"	Began before 18. Apparently gave up more than once. Last resumed 2 years ago	5	...
M-T-9*a*	44	A	7 cigarettes a day	Began at 18. Reduced consumption gradually after marriage	10	...
M-T-9*b*	44	A	About 15 cigarettes a day	Began at 18. Gave up for 6 weeks after operation in 1952	10	...
M-T-10*a*	39	C	15 cigarettes a week	Began at 16 or 17	9	...
M-T-10*b*	39	C	About 50 cigarettes a week	Began at 16. Reduced by one-third after marriage. Gave up for 8 and 6 weeks in 1942 and 1955 respectively during illness	10	...
M-T-11*a*	42	C	24 cigarettes (Reval *) or more and some cigars a day	Began at 14	17	...
M-T-11*b*	42	C	12 cigarettes (Reval) a day: 18 on Sundays (*Note*: M-T-11*a* also replied on behalf of M-T-11*b*)	Began at 14	10	...
M-T-12*a*	48	C	10 cigarettes a day	Began at 16	10	...
M-T-12*b*	48	C	About 12 cigarettes a day	Began at 24. Formerly smoked less. Smokes more now as result of business worries. Gave up for 6 months in 1944 as result of war wounds	10	...

* Reval is a particular brand of cigarettes

Monozygotic twins—continued

Number	Age at 1.1.58	Proximity of residence	Smoking habits at time of enquiry	Past smoking history	Classification of smoking habits	Blood groups and factors
M-T-13*a*	49	B	10 cigarettes a day	Began at 32	10	...
M-T-13*b*	49	B	10 cigarettes a day	Began at 17	10	...
...	82	B	2-3 cigars a day	Began at 20	15	...
...	Died 24.1.56	B	Smoked 10 cigars a day (*Note:* Information provided by his twin)	Began at 20	...	...
M-T-14*a*	41	C	About 8-10 cigarettes a day	Began at 17. Smoked more during war, gave up for 5 weeks in 1954 owing to operation, then resumed smoking gradually increasing to present level	10	...
M-T-14*b*	41	C	5-6 stumpen a day	Began at 24. Gave up temporarily for 4 weeks on account of illness	14	...
M-T-15*a*	38	C	8-10 cigarettes a day since 1955	Formerly smoked less	10	...
M-T-15*b*	38	C	Non-smoker	Smoked from 18 but gave up in 1945 when POW	4	...
M-B-1*a*	77	C	Non-smoker	Never smoked	1	...
M-B-1*b*	77	C	Non-smoker	Never smoked	1	...
M-B-2*a*	37	C	20 cigarettes a day	Began at 18. Smoked more after marriage	10	...
M-B-2*b*	37	C	20 cigarettes a day (*Note:* M-B-2*a* provided information about M-B-2*b* who is normally resident in U.S.A., but happened to visit his twin at the time of enquiry)	Began at 18. Smoked more after marriage	10	...
M-B-3*a*	45	B	25-30 cigarettes a day	Began at 16. Gave up temporarily in 1955 during illness	11	...
M-B-3*b*	45	B	20-25 cigarettes a day	Began at 15. Gave up 1945-46 for lack of funds	10	...

Monozygotic twins—continued

Number	Age at 1.1.58	Proximity of residence	Smoking habits at time of enquiry	Past smoking history	Classification of smoking habits	Blood groups and factors
M-B-4*a*	44	C	½ packet pipe tobacco (= 50 grams) a week	Began at 16. Smoked less after marriage. Sometimes gives up to economise	16	...
M-B-4*b*	44	C	12 cigarettes a day (*Note:* Information about M-B-4*a* provided by M-B-4*b*)	Began at 16. Smoked more in war	10	...
M-B-5*a*	44	C	50 grams of tobacco a week in form of hand-rolled cigarettes	Began at 18. Gave up for six months in 1955 during illness	12	...
M-B-5*b*	44	C	6 cigarettes a day	Began at 16. Gave up or reduced on occasions. Smoked less after marriage	10	...
M-B-6*a*	36	B	Non-smoker	Never smoked	1	...
M-B-6*b*	36	B	3-4 cigarettes a day	Began at 23. Gave up on occasions on health grounds. May smoke more in company or during intense mental work	9	...
M-B-7*a*	29	A	8-12 cigarettes a day	Began at 18. Gave up temporarily during severe illness	10	A_1B M Rh+
M-B-7*b*	29	A	12-15 cigarettes a day increasing to 25-30 when working hard mentally. Smokes occasional cigar	Began at 19. Twice gave up for 3 and 7 months respectively. Gives up during illness	10	A_1B M Rh+
M-B-8*a*	55	B	About 20 cigarettes a day	Began at 28	10	...
M-B-8*b*	55	B	Non-smoker	Never smoked	1	...
M-B-9*a*	25	A	Mainly pipe-smoker—3-4 average size pipes and 4-6 cigarettes a day	Began at 18. Gave up for 6 weeks in 1954	18	A MN
M-B-9*b*	25	A	About 8-10 cigarettes a day	Began at 21	10	A MN
M-B-10*a*	27	A	20-25 cigarettes a year, only in company	Began at 16	5	...
M-B-10*b*	27	A	20-25 cigarettes a year, only in company (Both twins described themselves as "smokers")	Began at 16	5	...

Monozygotic twins—continued

Number	Age at 1.1.58	Proximity of residence	Smoking habits at time of enquiry	Past smoking history	Classification of smoking habits	Blood groups and factors
M-B-11*a*	56	B	1 cigar a day	Began at 18	15	...
M-B-11*b*	56	B	Non-smoker	5 cigarettes daily until 1951 ; began at 18. Gave up on health grounds	2	...
M-B-12*a*	24	A	About 80 cigarettes a week	Began at 16	10	...
M-B-12*b*	24	A	Up to 10 cigarettes a day	Began at 16. Gave up once when ill	10	...
M-B-13*a*	27	B	Up to 10 cigarettes a day	Began at 18	10	O N rh−
M-B-13*b*	27	B	Up to 10 cigarettes a day	Began at 18 ; gave up from time to time for health reasons. Smoked less after marriage	10	O N rh−
M-B-14*a*	25	A	6 cigarettes a day	Began at 18. Gives up when ill	10	...
M-B-14*b*	25	A	5 cigarettes a day	Began at 18. Gives up when ill	10	...
M-B-15*a*	32	A	Non-smoker	Never smoked	1	A_1 MN rh−
M-B-15*b*	32	A	Non-smoker	Never smoked	1	A_1 MN rh−
M-B-16*a*	35	C	10-15 cigarettes a day	Began at 17	10	...
M-B-16*b*	35	C	1-2 cigars a day ; 50 grams tobacco every 3 or 4 days	Began at 21. Smoked very little in 1947-48 for economic reasons ; stopped temporarily in 1954 after operation	19	...
M-B-17*a*	61	B	60-80 cigarettes a week	Began at 17	10	A_1 MN Rh+
M-B-17*b*	61	B	15-20 cigarettes a day	Began at 15. Gives up when ill	10	A_1 MN Rh+
M-B-18*a*	68	B	60 cigarettes a week	Began at 31	10	...
M-B-18*b*	68	B	15 cigars a week	Began at 20	15	...
M-B-19*a*	31	B	Non-smoker	Never smoked	1	O MN
M-B-19*b*	31	B	Non-smoker	Never smoked	1	O MN

Monozygotic twins—continued

Number	Age at 1.1.58	Proximity of residence	Smoking habits at time of enquiry	Past smoking history	Classification of smoking habits	Blood groups and factors
M-B-20*a*	28	A	100 cigarettes a week	Began at 19	10	O N Rh+
M-B-20*b*	28	A	Non-smoker	Began at 23 ; smoked 2 cigars and 12 cigarettes a day until 6 months ago when he gave up for health reasons	3	O N Rh+
M-B-21*a*	27	A	Non-smoker	Never smoked	1	...
M-B-21*b*	27	A	Non-smoker	Never smoked	1	...
M-F-1*a*	36	B	15 cigarettes a day (120 a week)	Began at 18. Smoked more during war. Smokes less during illness	10	A N
M-F-1*b*	36	B	3-4 cigarettes a day	Began at 19	9	A N
M-F-2*a*	44	B	5-8 cigarettes a day	Began at 18. Smoked less during an illness	10	AB M
M-F-2*b*	44	B	3-4 cigarettes a day ; more at weekends	Began at 17	9	AB M
M-F-3*a*	67	C	Non-smoker	Began at 17 ; formerly smoked 10-15 cigarettes a day ; gave up 9 months ago after operation	2	A MN
M-F-3*b*	67	C	10-20 cigarettes a day	Began at 17	10	A MN
M-F-4*a*	26	B	10 cigarettes a day	Began at 16	10	A N
M-F-4*b*	26	B	10 cigarettes a day	Began at 16	10	A N
M-F-5*a*	51	B	1 cigar a day (5-6 a week)	Began at 18	15	A MN
M-F-5*b*	51	B	6 cigarettes a day	Began at 17-18. Smoked less during war. Gives up when ill	10	A MN
M-F-6*a*	27	B	Non-smoker	Never smoked	1	O MN
M-F-6*b*	27	B	Non-smoker	Never smoked	1	O MN

Monozygotic twins—continued

Number	Age at 1.1.58	Proximity of residence	Smoking habits at time of enquiry	Past smoking history	Classification of smoking habits	Blood groups and factors
M-F-7*a*	26	A	Non-smoker	Never smoked	1	O N
M-F-7*b*	26	A	Non-smoker	Never smoked	1	O N
M-F-8*a*	40	C	Non-smoker	Never smoked	1	O MN
M-F-8*b*	40	C	Non-smoker	Began at 17 to smoke in company ; gave up in 1948 after illness	4	O MN
M-F-9*a*	44	A	2-3 cigarettes a day ; 10 a day at weekends	Began at 20. Gives up when ill	9	O MN
M-F-9*b*	44	A	4 cigarettes a day (30 a week)	Began at 16. More during war ; gives up when ill	9	O MN
M-F-10*a*	24	A	Non-smoker	...	1	A_2 MN rh−
M-F-10*b*	24	A	Non-smoker	...	1	A_2 MN rh−
M-F-11*a*	29	C	18 cigarettes a day	Began at 16. Gave up from 1949-54. Re-started and gradually increased consumption	10	B MN
M-F-11*b*	29	C	12 cigarettes a day	Began at 25. Often gives up for short time	10	B MN
M-F-12*a*	23	A	Non-smoker	Never smoked	1	...
M-F-12*b*	23	A	Non-smoker	Never smoked	1	...
M-F-13*a*	23	A	10 cigarettes a day	Began at 18	10	A_2 M Rh+
M-F-13*b*	23	A	8 cigarettes a day	Began at 17. Gives up during illness	10	A_2 M Rh+
M-F-14*a*	50	A	10 cigarettes a day	Began at 18	10	O MN Rh+
M-F-14*b*	50	A	Non-smoker	Always a non-smoker	1	O MN Rh+
M-F-15*a*	28	A	15-20 cigarettes a day	Began at 21. Smoked more after marriage	10	A MN
M-F-15*b*	28	A	10-12 cigarettes a day	Began at 20. Smoked more after marriage	10	A MN
M-F-16*a*	30	B	10-12 cigarettes a day ; an occasional pipe	Began at 15-16	10	A MN
M-F-16*b*	30	B	About 12 cigarettes a day	Began at 16	10	A MN

Dizygotic twins

M-F-10*b*	36	B	About 12 cigarettes a day	Began at 16	10	A MN

Dizygotic twins

Number	Age at 1.1.58	Proximity of residence	Smoking habits at time of enquiry	Past smoking history	Classification of smoking habits	Blood groups and factors
D-T-1*a*	38	B	15 cigarettes a day when funds permit	Began at about 20	10	...
D-T-1*b*	38	B	About 10-15 cigarettes a day	Began at 23	10	...
D-T-2*a*	54	C	6-12 cigarettes a day ; a pipe on rare occasions	Began at 18. Smoked more during war when supplies available	10	...
D-T-2*b*	54	C	Non-smoker	Began at 20 ; 1-5 cigarettes a day ; gave up at age of 50 on health grounds. Smoked more during war	2	...
...	Died 24.4.57	C	...	...	...	...
...	52	C	10-12 cigarettes a day	Began at 19	10	...
D-T-3*a*	34	C	6 cigarettes a day	Began at 19. Smoked more during war	10	...
D-T-3*b*	34	C	Non-smoker	Began at 18 ; gave up on health grounds at date not specified	4	...
D-T-4*a*	35	A	Non-smoker	Began at 20 ; gave it up in 1948 when he was smoking 25 cigarettes a day on health and economic grounds	2	...
D-T-4*b*	35	A	12 cigarettes a day ; 20 a day on rest days	Began at 18. Gave up when ill	10	...
D-B-1*a*	43	C	2 packets (50 grams) of pipe tobacco a week and 3 cigars on Sundays ; cigarettes very rarely	Began at 15. Smoked 40-60 cigarettes a day during war until taken POW. Smoked more after marriage	19	$A_{2(1)}$ MN rh−
D-B-1*b*	43	C	4 cigarettes and 2 pipes a day ; no cigars	Began at 18	18	O M Rh+
D-B-2*a*	36	A	Non-smoker	...	1	...
D-B-2*b*	36	A	12 cigarettes a day	Began at 14. Smoked less during war and post-war years for economic reasons	10	

Dizygotic twins—continued

Number	Age at 1.1.58	Proximity of residence	Smoking habits at time of enquiry	Past smoking history	Classification of smoking habits	Blood groups and factors
D-B-3*a*	25	A	Non-smoker	Never smoked	1	...
D-B-3*b*	25	A	Non-smoker	Never smoked	1	...
D-B-4*a*	29	A	6 cigarettes a day	Began at 17	10	B
D-B-4*b*	29	A	2 cigarettes a day	Began at 20; did not smoke in 1952 because of illness	9	B
D-B-5*a*	26	B	" Non-smoker "	Began at 20 as " occasional smoker up to to-day "	8	B M
D-B-5*b*	26	B	6-9 cigarettes a day	Began at 18. Gives up temporarily for health or economic reasons	10	O MN
D-B-6*a*	24	A	6-8 cigarettes a day	Began at 18	10	...
D-B-6*b*	24	A	12 cigarettes a day and a packet of pipe tobacco weekly	Began at 17	18	...
D-B-7*a*	28	B	About 10 cigarettes a day and/or 2-3 packets of cigarette tobacco a week	Began at 18. Gives up temporarily for health or economic reasons. Smoked slightly more after marriage	13	...
D-B-7*b*	28	B	About 12 cigarettes a day	Began at 16. Smoked more after marriage	10	...
D-B-8*a*	34	C	Non-smoker	Never smoked	1	...
D-B-8*b*	34	C	Non-smoker	Never smoked	1	...
D-B-9*a*	23	A	Non-smoker	Never smoked	1	...
D-B-9*b*	23	A	Non-smoker	Never smoked	1	...
D-B-10*a*	21	A	Non-smoker	Never smoked	1	...
D-B-10*b*	21	A	Non-smoker	Never smoked	1	...
D-B-11*a*	21	A	1-2 cigarettes a day in company	Began at 18	9	...
D-B-11*b*	21	A	Non-smoker	...	1	...

Dizygotic twins—continued

D-B-11*b*	21	A	Non-smoker	...	...	...

Dizygotic twins—continued

Number	Age at 1.1.58	Proximity of residence	Smoking habits at time of enquiry	Past smoking history	Classification of smoking habits	Blood groups and factors
D-F-1*a*	26	A	Non-smoker	Never smoked	1	A N
D-F-1*b*	26	A	Non-smoker	Never smoked	1	A N
D-F-2*a*	22	A	12 cigarettes a day (■4 a week)	Began at 18 ; gave up for 6 months on one occasion following operation	10	A N
D-F-2*b*	22	A	3-4 cigarettes a week. Describes himself as an " occasional smoker "	Began at 18	5	A N
D-F-3*a*	31	C	30 cigarettes a week and a packet of pipe tobacco fortnightly	Began at 16 ; stopped for 6 weeks in 1944 following operation	18	O M
D-F-3*b*	31	C	1 small packet of pipe tobacco a week	Began at 17 ; reduced consumption on marriage	16	O N
D-F-4*a*	35	A	3 cigars a day from 1957	Began at 18 ; smoked 10 cigarettes a day 1949-1956 prior to which he smoked less	15	A M
D-F-4*b*	35	A	3 cigarettes a day	Began at 19	9	A M
D-F-5*a*	20	A	50 grams of pipe tobacco and 10 cigarettes a week	Began at 18. Occasionally gave up for 1-2 months. Gives up during illness	18	AB MN
D-F-5*b*	20	A	Non-smoker	A " passionate " non-smoker	1	A N
D-F-6*a*	49	B	10 cigarettes a day	Began at 14	10	AB N
D-F-6*b*	49	B	Non-smoker	Never smoked	1	A MN
D-F-7*a*	29	B	Non-smoker	Began at 16 ; gave up in 1950 on health grounds	4	A N
D-F-7*b*	29	B	20-50 cigarettes a week, varies a lot	Began at 14 ; changed level of consumption from time to time on economic grounds	10	O N
D-F-8*a*	24	A	" Non-smoker " ; a pipe on rare occasions	Began at 14	7	...
D-F-8*b*	24	A	" Non-smoker " ; a cigar on rare occasions	Began at 15	6	...

Dizygotic twins—continued

Number	Age at 1.1.58	Proximity of residence	Smoking habits at time of enquiry	Past smoking history	Classification of smoking habits	Blood groups and factors
D-F-9*a*	21	B	3 cigars a day, 15 a week	Began at 16	15	O M
D-F-9*b*	21	B	12 cigarettes a day	Began at 14	10	O M
D-F-10*a*	23	A	6 cigarettes a day	Began at 18	10	...
D-F-10*b*	23	A	Non-smoker	...	1	...
			(*Note:* Information for both twins provided	by their father		
D-F-11*a*	26	C	6-12 cigarettes a day	Began at 20	10	A MN
D-F-11*b*	26	C	Non-smoker	Began at 17, gave up in 1953	4	B MN
D-F-12*a*	50	C	10 stumpen a day	Began at 23	14	O N
D-F-12*b*	50	C	2 cigars a day	Began at 30	15	O MN
D-F-13■	78	B	10 cigars a day and 100-150 grams pipe tobacco a week	Began at 17-18. Smoked less during war	19	O MN
D-F-13*b*	78	B	150 grams pipe tobacco a week	Began at 18. Prior to 1914 smoked mainly cigars, thereafter mainly a pipe with cigars now and then	16	O MN
D-F-14*a*	36	B	Non-smoker	Never smoked	1	A M
D-F-14*b*	36	B	20 cigarettes a day	Began at 16	10	A M
D-F-15*a*	25	A	10-15 cigarettes a day	Began at 16	10	A MN
D-F-15*b*	25	A	Non-smoker	Began at 15 ; smoked 10 cigarettes a day until recently when he gave up for "common-sense reasons"	2	A M
D-F-16*a*	30	A	About 6 cigarettes a day	Began at 18	10	A MN
D-F-16*b*	30	A	Non-smoker	Never smoked	1	O MN
D-F-17*a*	49	C	"A non-smoker"—rarely a cigarette or cigar (preferably the latter) in company	...	6	A MN
D-F-17*b*	49	C	"More a non-smoker than a smoker"	Began during the war but never smoked much. Average of 1 cigarette or 1 cigar a week. Smoked more during the war	6	A N

SELECTION AND POPULATION STRUCTURE IN *LOLIUM*

II. GENETIC CONTROL OF DATE OF EAR EMERGENCE

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1. INTRODUCTION

A COMPARATIVE study of the heading behaviour of three contrasting ryegrass strains, Wimmera, Irish and Kent (Cooper, 1959) showed that in addition to the broad differences between the strains, appreciable genetic variation occurred between plants within each strain. These differences in heading behaviour could be traced to genetic differences in both " winter requirement " for short-day or cold before floral induction, and also the need for long photoperiods for floral initiation. Previous studies in the outbreeding species of *Lolium* (Cooper, 1954) had shown that both these responses were under polygenic control, and that most parents were heterozygous for genes controlling flowering responses.

In selecting for any character, two parameters are important ; firstly, the amount of genetic variation in the population, and secondly, the precision with which it is transmitted, *i.e.* the heritability. These together give a measure of the possible rate of change under selection, since the expected genetic gain is the product of the heritability and the selection differential.

The present paper attempts to estimate the genetic variation in date of ear emergence within two perennial ryegrass populations, Irish and Kent, to calculate its heritability and hence to predict the potential advance under selection. The genetic control of the " winter requirement " for floral induction has also been studied and will be reported later.

The genetic variation has been assessed in two ways ; firstly, from the analysis of replicated clones, both within and between years, providing an estimate of the environmental and the *total* genetic variation ; and secondly, by the regression of progeny means on mid-parent values, thus distinguishing the *additive* portion of the genetic variance, *i.e.* that part which is fixable by selection. In Mather's notation (Mather, 1949), this total genetic variance of the population can be denoted by $\frac{1}{2}D + \frac{1}{4}H$, where D is the additive portion due to differences between alleles at the same locus, and H is that due to dominance.

2. ANALYSIS OF REPLICATED CLONES

For a character such as ear emergence, where the initial size of the plantlet is not critical, a separation of environmental and *total* genetic variation is readily obtained by the use of clonal replicates. A number of clones of Irish and Kent ryegrass were therefore planted out in replicated blocks in the field in the autumn of 1954, 1955 and 1956, and the date of ear emergence was recorded for each plant the following spring. The exact dates of planting are given in table 1.

TABLE 1

Sowing and planting dates of seedlings and clones

Year recorded	Seedlings		Clones planted
	Sown	Planted	
1954	28 Aug. 53	29 Sept. 53	...
1955	23 Aug. 54	12 Oct. 54	18 Oct. 54 *
1956	13 Aug. 55	13 Oct. 55	13 Oct. 55
1957	13 Aug. 56	25 Oct. 56	16 Nov. 56

* In pots outdoors.

(a) *Within years*

The components of variation due to genotype, block and error (including block-genotype interaction) were assessed within each year as shown in table 2. In 1955, only two replicates of each genotype were available and these were randomised within one block, so block differences cannot be calculated for that year. The 1956 and 1957 material also included clones derived from single crosses and selfings within the original populations. The results for these clones are given separately.

Over the three years, the error variance of a single spaced plant varied from 1·25 to 4·58 days, and this is of the same order as found earlier for homozygous lines of *Lolium temulentum* (Cooper, 1954). There were no regular differences between Irish and Kent, or between the original population plants and the progeny derived from them by pair-crossing or selfing. The variation due to blocks was very low, and by far the greatest portion of the variation within the strains was genetic. The proportion of the total variation attributable to genetic differences between clones, giving a measure of heritability in the broad sense is above 0·85 in all cases. The genetic variance of the groups from pair-crosses and selfings is higher than that of the original populations, since these groups included plants deliberately selected for extremely early and extremely late ear emergence.

TABLE 2

TABLE 2

Date of ear emergence: Partition of variation from replicated clones 1955-57

	Irish								Kent							
	Mean	V_g	V_b	V_e	V_t	V_g/V_t	Number of		Mean	V_g	V_b	V_e	V_t	V_g/V_t	Number of	
							Clones	Blocks							Clones	Blocks
1955																
Original population	11·4 May	72·87	...	2·75	75·62	0·964	24	2	27·6 May	37·98	...	1·25	39·23	0·968	16	2
1956																
Original population	10·9 May	27·91	0·35	2·86	31·22	0·894	24	5	25·6 May	66·00	0·60	3·02	69·62	0·948	15	5
Crosses . . .	12·7 May	58·06	0·25	2·23	60·54	0·959	16	5	25·9 May	160·08	0·47	2·86	163·41	0·979	14	5
Selfings . . .	12·9 May	62·89	0·13	3·88	66·90	0·940	15	5	1·7 June	149·42	0·16	3·36	152·94	0·977	8	5
1957																
Original population	1·9 May	28·09	0·12	4·25	32·46	0·865	38	5	24·9 May	89·60	−0·04	2·93	92·49	0·969	34	5
Crosses . . .	6·9 May	111·93	0·86	4·58	117·37	0·945	8	5	23·6 May	203·18	0·08	3·12	206·38	0·984	15	5
Selfings . . .	4·5 May	95·56	0·25	3·31	99·12	0·954	9	5	2·6 June	204·75	0·03	3·09	207·27	0·988	5	5

Analysis of variance, Irish 1956

	dF	SS	MS	Expectation
Genotypes . .	23	3276·2	142·4	V_e+5V_g
Blocks . . .	4	45·3	11·3	V_e+24V_b
Error . . .	92	263·5	2·86	V_e
Total . .	119	3585·0	...	

(b) *Between years*

The above estimates of genetic variation were calculated for each year independently, but it is important to know the " repeatability " of this character from year to year. It has been shown earlier (Cooper,

TABLE 3

Date of ear emergence : Correlation between years for clonal plants

	Mean			Correlation			No. of clones
	1955	1956	1957	55/56	55/57	56/57	
Irish Outbred .	11·0 M	10·0 M	4·5 M	+0·938	+0·862	+0·940	24
Selfed . .	18·8 M	13·0 M	7·5 M	+0·935	+0·956	+0·819	14
Kent Outbred .	27·8 M	25·8 M	23·5 M	+0·861	+0·912	+0·944	16
Selfed . .	1·9 J	31·1 M	29·5 M	+0·934	+0·917	+0·923	10

1959) that Irish perennial ryegrass shows considerable variation between years and that most of this variation can be attributed to spring temperatures during inflorescence development. In the present work, a number of clones of Irish and Kent ryegrass were recorded in

TABLE 4

Date of ear emergence : Genotype-year interaction 1956-57

	V_g	V_y	V_{gy}	V_e	V_t	V_g/V_t	No. of clones
Irish . . .	52·7	17·0	2·5	3·6	75·8	0·695	24
Kent . . .	119·4	4·5	5·3	3·0	132·2	0·903	27
Combined . .	154·8	9·5	4·9	3·3	172·5	0·897	51

Analysis of variance : Irish

	dF	SS	MS	Expectation
Genotypes . . .	23	12,505	543·7	$V_e+5V_{gy}+10V_g$
Years . . .	1	2,059	2059·0	$V_e+5V_{gy}+120V_y$
Genotype/Year . .	23	375	16·3	V_e+5V_{gy}
Genotypes within years .	47	14,939	317·9	
Error . . .	192	687	3·6	V_e
Total . . .	239	15,626		

all three years, 1955 to 1957. The mean dates of ear emergence and the correlations between years are shown in table 3, while fig. 1 illustrates the correlation between one early year (1957) and one late year (1956).

The correlations between years were high and did not differ significantly between the Irish and Kent clones, nor between the outcrossed and inbred material, although date of ear emergence shows marked inbreeding depression and the selfed plants were invariably later than the corresponding outcrosses.

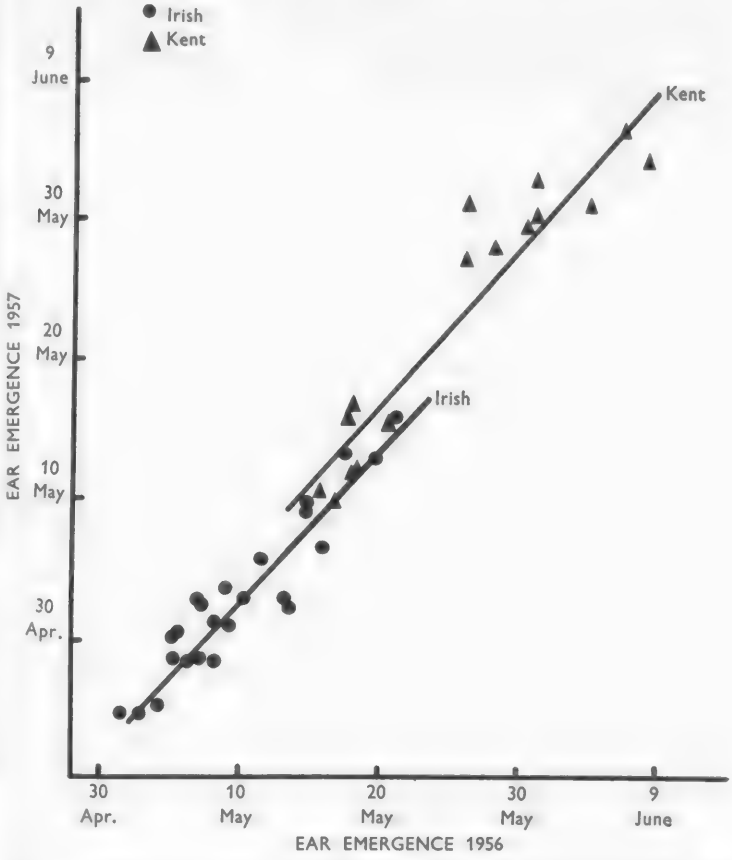

FIG. 1.—Correlation between years : clonal replicates of Irish and Kent ryegrass. 1956 and 1957.

A more detailed analysis of the extent of genotype/year interaction is presented in table 4, for one late year (1956) and one early year (1957). In both Irish and Kent, significant interaction occurs, but it is small compared to differences between genotypes. The difference between the two years is large in Irish, but smaller in Kent.

In conclusion, it can be said that most of the variation within

populations is genetic, and that different genotypes maintain the same relative order of expression from year to year.

3. PARENT-PROGENY REGRESSION

While the analysis of replicated clones allows of the separation of environmental and *total* genetic variation, in order to distinguish between *additive* and *non-additive* genetic variation a breeding test is necessary. In the present work, records from both pair-crosses and selfings have been used. The exact sowing and planting dates are given in table 1.

TABLE 5

Degree of assortative mating: Correlation between parents of pair-crosses

	Irish		Kent	
	r	No. of families	*r*	No. of families
1955	+0·749	9	+0·873	9
1956	+0·924	16	+0·934	14
1957	+0·553	16	+0·653	7

The crosses were made according to the mutual pollination method of Jenkin (1931*a*), using positive assortative mating for date of ear emergence. The degree of assortative mating, as measured by the correlation between parents, is given in table 5. It is high in 1955 and 1956 but lower in 1957. Such mutual pollination without emasculation means that some self-pollination can occur, but the degree of self-fertility is very low in most plants. Table 6 gives estimates for the Irish and Kent ryegrass used in the present work, together with combined data from Jenkin (1931*b*) on ryegrass from various sources. Furthermore, the positive assortative mating and consequent high correlation between parents will decrease the effect of an occasional selfing on either the means or variances of the progenies. In a comparison in 1955 (see table 8), the variance of selfed progenies was of the same order as that of corresponding pair-crosses.

The heritability of date of ear emergence has been assessed from these crosses by parent-progeny regression and correlation. While the regression coefficient measures the linear relation between parents and progeny, the correlation coefficient indicates the amount of scatter around this line. The maximum value for the regression of progeny on an individual parent ranges from $\frac{1}{2}$ under random mating to 1 under complete genetic assortative mating (*i.e.* selfing). Incomplete assortative mating will fall between these limits. In the present

calculations, however, the difficulty of allowing for the degree of assortative mating has been overcome by using the regression of the progeny mean on the mid-parent value, which has a maximum value of 1, irrespective of the breeding system. Estimates are given in

TABLE 6

Self-fertility in Lolium perenne

Seeds per 100 spikelets	Percentage of plants		
	Irish	Kent	Data from Jenkin (1931*b*)
0-5 . . .	37·6	25·0	50·0
6-10 . . .	8·1	15·0	19·0
11-15 . . .	12·1	10·0	6·0
16-20 . . .	4·4	0·0	6·0
21-50 . . .	24·5	35·0	14·0
51-100 . .	12·0	10·0	3·0
More than 100 .	1·3	5·0	2·0
	(66 plants)	(22 plants)	...

table 7 for pair-crosses of Irish and Kent in 1955, 1956 and 1957, and for selfings in 1955 only, while fig. 2 illustrates the behaviour of pair-crosses and selfings in 1955, and fig. 3 that of pair-crosses only in 1956.

TABLE 7

Heritability of date of ear emergence 1955-57

	Irish			Kent			Combined		
	b	r	No.	b	r	No.	b	r	No.
1955									
Crosses * .	0·72±0·062	+0·98	9	0·94±0·082	+0·98	9	0·87±0·049	+0·98	18
Selfings * .	0·64±0·045	+0·98	8	0·96±0·013	+0·95	7	0·82±0·059	+0·97	15
1956									
Crosses .	0·79±0·063	+0·96	15	0·83±0·060	+0·96	14	0·87±0·033	+0·98	30
1957									
Crosses .	0·87±0·186	+0·78	16	0·97±0·157	+0·94	7	1·04±0·058	+0·97	23

All progeny means based on 50 seedlings.

* Parents in pots in gardens, progeny in field.

Except for 1955, the regression coefficients for Irish and Kent are similar and close to 1·0, but there is a tendency for the progeny from both extremely early and extremely late parents to approach closer to the population mean.

In 1955, the parent clones were grown in pots in the experimental garden, while the progenies were put out as spaced plants in the field. The high spring temperatures in the garden resulted in earlier heading in the parent clones, the acceleration being greater in the earlier

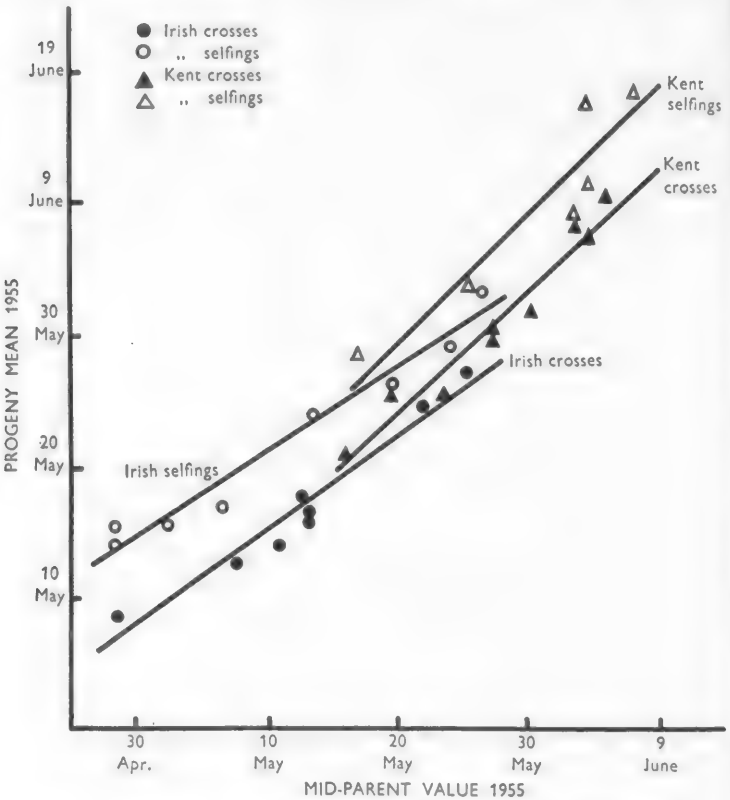

FIG. 2.—Heritability of date of ear emergence in Irish and Kent ryegrass. 1955.

Irish crosses	$Y = 0{\cdot}72X + 7{\cdot}76$
,, selfings	$Y = 0{\cdot}64X + 14{\cdot}65$
Kent crosses	$Y = 0{\cdot}94X + 5{\cdot}17$
,, selfings	$Y = 0{\cdot}96X + 10{\cdot}39$

material. The regression of progeny on mid-parent value was therefore rather lower (0·72 and 0·64) in this year for the early-flowering Irish but remained high for Kent ryegrass. This effect is well shown if the results for 1955 (fig. 2) are compared with the corresponding date for 1956 (fig. 3) when all parents and progeny were grown in the field.

These high regression coefficients indicate that most of the genetic variation is additive. Their absolute values will not have been affected by the positive assortative mating for ear emergence, but their accuracy of estimation will have been increased.

The correlations between mid-parent and progeny means are above 0·9, except for Irish crosses in 1957, with no apparent difference between Irish and Kent or between crosses and selfings. The parent and progeny means, however, show an inbreeding depression on selfing of about 5-6 days in each strain.

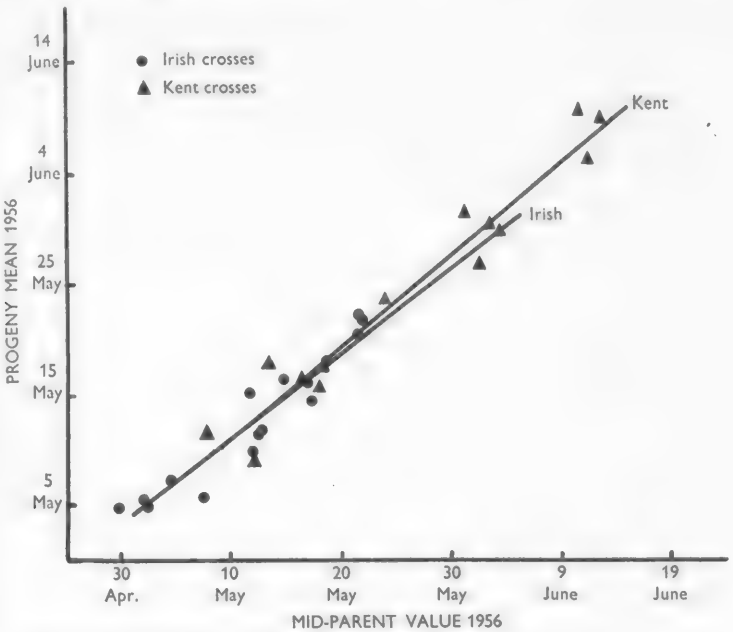

FIG. 3.—Heritability of date of ear emergence in Irish and Kent ryegrass. 1956.

Irish crosses $Y = 0{\cdot}79X + 3{\cdot}03$
Kent crosses $Y = 0{\cdot}83X + 2{\cdot}76$

In conclusion, these data from pair-crosses and selfings indicate that date of ear emergence is highly heritable in both populations, but is affected by inbreeding depression. Most of the genetic variance is additive, and considerable response to selection should be possible.

4. VARIATION WITHIN PROGENIES FROM PAIR-CROSSING AND SELFING

In spite of the high heritability of date of ear emergence as calculated from parent-progeny regression, the results show that most parents

from the original populations are rather heterozygous for genes controlling this character. This is illustrated in table 8 which gives the means and variances of the original populations, of the parents used for crossing, and the means, mean variance, and the variance of means of their progenies for the three years, 1955 to 1957. The table

TABLE 8

Date of ear emergence : Variation within progenies from pair-crosses and selfings

	Irish			Kent		
	1955	1956	1957	1955	1956	1957
Original populations						
Mean of means	17·4 M	7·3 M	28·9 A	1·7 J	24·4 M	22·3 M
Mean var.	29·8	12·9	22·5	24·5	26·5	44·6
No. of lots	2	13	6	1	6	6
Pair-crosses						
Parents—						
Mean	12·0 M*	12·4 M	2·0 M	27·6 M*	26·6 M	24·8 M
Var.	67·3	54·7	23·6	47·2	150·4	54·4
Progenies—						
Mean of means	16·5 M	12·8 M	27·5 A	31·0 M	24·8 M	22·0 M
Mean var.	21·1	12·3	17·7	10·8	23·0	17·2
Var. of means	37·0	36·9	29·4	43·6	109·7	57·9
No. of families	9	16	16	9	14	7
Selfings						
Parents—						
Mean	11·2 M*	...	...	28·6 M*	...	...
Var.	127·5	...	...	74·2	...	...
Progenies—						
Mean of means	21·6 M	...	...	6·9 J	...	...
Mean var.	21·6	...	...	31·1	...	...
Var. of means	49·0	...	...	76·0	...	...
No. of families	8	...	...	7	...	...
Error variance of single plant (E_1)	2·75	2·86	4·25	1·25	3·02	2·93
Error variance of mean of 50 (E_2)	0·055	0·057	0·085	0·025	0·060	0·059

* Parents in pots in gardens.

Progeny means and variances based on 50 plants.

also includes estimates, derived from replicated clones, of the error variance of single spaced plants, (E_1) and of the means of 50 (E_2).

The means of the populations varied from year to year but their variances were of the same order, ranging from 12·9 to 44·6. In all cases, the variance of the parental group was larger than that of the original population, the result of using some very early and very late plants as parents.

In pair-crosses under random mating, both the genetic variance of the progeny means and the mean genetic variance of the progeny would be expected to be about half that of the parental group, since, in Mather's notation (Mather, 1949) :

$$\text{Variance of parental group} = \tfrac{1}{2}D+\tfrac{1}{4}H+E_1$$
$$\text{Mean variance of progenies} = \tfrac{1}{4}D+\tfrac{3}{16}H+E_1$$
$$\text{Variance of progeny means} = \tfrac{1}{4}D+\tfrac{1}{16}H+E_2$$

Under assortative mating, however, as shown by Breese (1956), while the mean variance of the progenies remains comparatively unaffected, the variance of progeny means is considerably increased, approaching in the extreme case that expected under selfing ($\frac{1}{2}D+\frac{1}{16}H+E_1$). In the present results under such assortative mating, the variance of progeny means is always well above that expected on the basis of random mating, when compared to the parental group.

In the selfings, which were available in 1955 only, the parental group also showed a higher variance than the original population. In this case, the expected progeny variances are as follows :

$$\text{Mean variance of progenies} = \tfrac{1}{4}D+\tfrac{1}{8}H+E_1$$
$$\text{Variance of progeny means} = \tfrac{1}{2}D+\tfrac{1}{16}H+E_2$$

In accordance with this expectation, the variance of progeny means is rather larger for the selfings than for the pair-crosses, even with assortative mating, while, for Irish at least, the mean variances are of the same order.

Even so, the mean variance from crossing two plants of similar phenotype, or from selfing one, is well above the error variance of a single clone, indicating considerable heterozygosity of the parents. The mean variances of the Irish and Kent progenies are of the same order, suggesting a similar degree of heterozygosity, or storage of variation, within plants from the two populations.

The comparatively small variance of the original populations, not much greater than that within a single pair-cross, suggests that selection for uniformity of heading within the seed crop is quite stringent. A single date of harvesting severely limits the range of parents which can be represented in the next generation. Plants which flower too early will lose seed by shedding, while plants which are too late may not ripen seed before harvest (Cooper, 1959). Such selection against extremely early and extremely late plants as female parents will be reinforced by the inevitable positive assortative mating for flowering date within the seed crop, so that extremely early and extremely late parents will also tend to be excluded as pollen parents.

In view of the complexities of this genetical situation which involve (i) non-random mating in the original population, (ii) heterozygosity of all parents, (iii) selection of parents to increase the variance of the parental group, and (iv) positive assortative mating for flowering

time, no attempt has been made to derive strict estimates of the components of variation, D, H and E from the present data. However, as shown in table 2, E is small and does not vary greatly between years, between strains, or between outcrossed and inbred material; while the high parent-progeny regressions indicate that most of the genetic variance is additive. Considerable response to selection should therefore be possible.

5. DISCUSSION

The estimation of genetic variation and heritability involves the analysis of the total variation into those portions due to genotype, to the environment, and to genotype/environment interaction. The genetic component can be further analysed into those parts resulting from additive gene action, from dominance, and from other gene interactions.

A preliminary separation of environmental from *total* genetic variation can be obtained from replicated clones, from homozygous lines or from the F_1s between them. In herbage species, replicated clones have been used by Burton and Devane (1953) and by Fejer (1958), while homozygous lines have been used by Burton (1952) and by Davern, Peak and Morley (1957). The variation within clones or within homozygous lines is taken as a measure of environmental variation, and the heritability in the broad sense is calculated as :

$$\frac{V_T - V_E}{V_T}$$

or, in Mather's notation : $$\frac{\frac{1}{2}D + \frac{1}{4}H}{\frac{1}{2}D + \frac{1}{4}H + E}$$

assuming that the sample is random, and is derived from a randomly-breeding population. A low heritability may therefore simply mean little genetic variation.

The estimation of the *total* genetic variation, however, only gives a maximum figure for the genetic variance and for the heritability. In predicting the potential response to selection, it is the *additive* (*i.e.* easily fixable) part of the genetic variance which is important, and this can only be assessed by a breeding test. Where homozygous parental lines are available, and both selfing and crossing are practicable, a number of estimates of genetic parameters are possible (Mather, 1949), and a recent refinement of these techniques is the analysis of diallel crosses, which allows of the separation of non-additive genetic variance into dominance and non-allelic gene interaction (Jinks, 1954, 1956; Dickinson and Jinks, 1956).

In the outbreeding perennial herbage grasses, however, homozygous material is not usually available and selfing is often difficult or impossible. A more usual procedure has therefore been to use the regression or correlation between parent and progeny, the progeny being obtained either by pair-crossing, selfing or open-pollination.

The use of such methods in the herbage grasses has been reviewed by Burton (1952) and by Fejer (1958). For pair-crosses, heritability in the narrow sense is estimated as the regression of the progeny mean on the mid-parent value, while for open pollination (assuming random mating) it is given by twice the regression of the progeny mean on the female parent.

In the assessment of heritabilities from pair-crosses, random mating has often been thought necessary, but Reeve (1953) points out that unless the situation is complicated by dominance and non-allelic interaction, the maximum heritability values can be obtained by (*a*) selecting parents to increase their variance above that of the original population, and (*b*) using positive assortative mating to increase the variance of the mid-parent value. Both these methods are combined in the usual type of selection experiment, and in most plant or animal breeding programmes.

In the present work, the methods employed have been determined by the outbreeding and largely self-incompatible nature of the crop, and it is interesting that the results obtained, although derived from simple regression analysis, agree well with those obtained by more refined methods in self-fertilising species. In *Nicotiana rustica*, for instance, Mather and Vines (1952) found that most of the genetic variance for flowering time was additive, that there was no interaction between years, and that the error variance of a single plant was about 6 to 10 days. This material was further studied by Jinks (1954, 1956) using a diallel analysis of the parents and F_1s which he later extended to the F_2 and backcross generations. From the parent and F_1 analysis he concluded that time of flowering showed incomplete though significant dominance, but a complete absence of non-allelic interaction, while the F_2 and backcross analyses showed only slight non-allelic interaction. Great seasonal differences occurred in the size of the genetic components of variation. In further analyses of the genetic/environment interaction, Allard (1956) suggested that for time of flowering the additive genetic effects were comparatively stable, but that dominance effects varied in different environments.

Similar conclusions are drawn by Davern, Peak and Morley (1957) from their analysis of flowering time in the self-fertilising *Trifolium subterraneum*. The repeatability of parental lines between years was high (86 per cent.), and no large block effects were noted. The regressions of the F_1 means on mid-parent values and of F_3 means on F_2 parents were not significantly different from unity, indicating that most of the genetic variation was additive.

The same general pattern is revealed in most studies on the genetic control of flowering time. Most of the genetic variation seems to be additive, with little dominance or non-allelic interaction. Selection in the past has presumably been for an intermediate phenotype, which would not be expected to lead to fixation of alleles, or to the development of directional dominance. Considerable response to

future selection should therefore be possible, and such has proved to be the case in the present ryegrass strains, Irish and Kent. The results of four years' selection for early and late ear emergence will be reported in the next paper, and the actual response to selection compared to that expected from the present estimates of additive genetic variance and heritability.

6. SUMMARY

1. This paper attempts to estimate the genetic variation for date of ear emergence within Irish and Kent ryegrass, to calculate its heritability, and hence predict the potential advance under selection.

2. The genetic variation has been assessed in two ways: (i) from the analysis of replicated clones, and (ii) from the regression of progeny means on mid-parent values.

3. Most of the variation within each population in any one year is genetic, block effects being small, and the repeatability between years is also high, although variation in absolute values occurs between early and late years.

4. Estimates derived from parent-progeny regression show that most of the genetic variation is additive, although marked inbreeding depression occurs on selfing, and that considerable response to selection would be expected for this character.

5. In spite of the high heritability of date of ear emergence, most parents from Irish and Kent ryegrass are highly heterozygous for genes controlling ear emergence, and the variance of progenies from selfing or pair-crossing is of the same order as that of the original population.

6. Methods for the estimation of genetic variation and heritability in herbage plants are discussed, and it is concluded that in other species also flowering time seems to show mainly additive genetic variance, with little or no dominance.

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SELECTION AND POPULATION STRUCTURE IN *LOLIUM*

III. SELECTION FOR DATE OF EAR EMERGENCE

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1. INTRODUCTION

In a previous paper (Cooper, 1959*b*) it was shown that in both Irish and Kent ryegrass most of the variation in date of ear emergence was genetic and additive, and that all the plants tested were highly heterozygous for genes controlling this character. Considerable response to selection should therefore be possible. The present paper describes the actual response within these two populations during the first three generations of selection for early and late heading. The experiment was designed to compare the effects of different mating systems and initial population sizes on the response to selection, and also to assess the amount of potential genetic variation carried within one plant or a small group of plants. In addition, during summer 1957, ear measurements were made on the initial populations, and on parents and progenies of all three generations to detect any correlated response to selection, and to assess the effect of different degrees of inbreeding on these characters. These results will be discussed in a later paper.

2. MATERIAL AND METHODS

The two initial populations of Irish and Kent ryegrass consisted of commercial samples from the same merchant. In each year, beginning in 1953, seedlings and clonal material of the parents of all generations were planted out in the autumn, and the date of ear emergence was recorded the following spring. Details of the sowing and planting dates have been given earlier (Cooper, 1959*b*). The plants to be used as parents in each year were lifted immediately after ear emergence, potted and removed to the greenhouse for crossing. In this way a generation time of one year was obtained. The initial selection in summer 1954 was made in the Agricultural Botanic Garden, University of Reading, but all subsequent generations were tested at the Welsh Plant Breeding Station, Aberystwyth.

(i) *Mating systems*

The first crosses were made in summer 1954. The earliest and latest four plants out of 125 were lifted from Irish and Kent ryegrass, each was selfed, and the plants within each group were crossed in pairs. These crosses were made without emasculation according to the mutual pollination method of Jenkin (1931). This may result in a small proportion of selfing but does not affect the present conclusions (Cooper, 1959*b*).

From these crosses, four main mating systems were established as shown in fig. 1, the aim being to derive early and late lines from each initial group, and so to assess the variation carried within one plant, within two, and within a group of four.

(*a*) *Four-plant lines* (*Fourway crosses*). In the first generation only pair-crosses were made between the four plants of each group, but in the next year, 1955, the earliest and the latest two plants were selected from two complementary pair-crosses within each group; the four early plants were allowed to intercross and also the four late ones. This gave rise to four selection lines from each strain, an early/early, early/late, late/early and late/late, denoted EE, EL, LE and LL respectively. This mating system was continued in subsequent generations by intercrossing the four extreme plants, either early or late, within each line. Each pair of lines is thus based on four unrelated plants (8 different chromosome sets) from the same population.

(*b*) *Two-plant lines* (*Sib-crosses*). In the second generation in 1955, the two complementary pair-crosses used to produce the fourway lines were each continued by sib-mating. The two earliest and two latest plants from each progeny were selected,

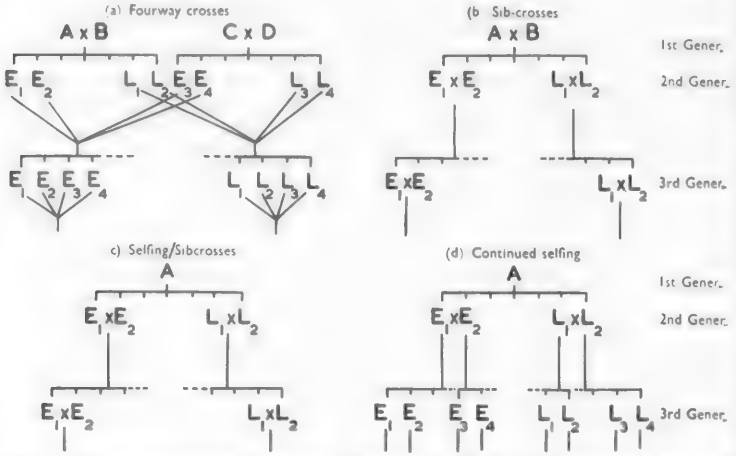

FIG. 1.—Mating systems used in selection experiment. (A, B, C, D represent the original parents of each selection group, while E_1, E_2 etc. and L_1, L_2 etc. represent the plants selected for earliness or lateness respectively in subsequent generations.)

and from these EE, EL, LE and LL lines were derived. Starting with two pair-crosses, this gave two lines of each kind from each strain. Each pair of lines is thus based on only two plants (4 different chromosome sets).

(*c*) *Single-plant lines* (*selfing followed by sib-mating*). Of the 8 initial parents of each strain selfed in 1954, only 4 of Irish and 5 of Kent produced more than 20 seedlings. From these, two early and two late progenies were selected in each strain, and from each progeny an early and a late line were derived by sib-mating. These lines were continued by sib-mating in subsequent generations, giving two lines of each type for each strain. Each pair of lines is thus based on only one plant (2 different chromosome sets).

(*d*) *Single-plant lines* (*continued selfing*). It was hoped to establish early and late lines from within the selfed progenies by continued selfing. Self-sterility and lack of vigour, however, made this impossible, although a few lines were maintained to the third generation.

In addition to these main mating systems, fourway crosses were made in 1956 between the two duplicate sib-mating lines and also between the two duplicate selfing/sib-mating lines within each selection group, to study the effect of relaxing inbreeding while maintaining the direction of selection.

The selection differentials and the coefficients of inbreeding for the different mating systems are given in table 1. The coefficients of inbreeding are calculated on the assumption that selfing does not occur in the fourway crosses or pair-crosses. The actual amount of selfing is small, and would result in only a slight increase in the coefficient of inbreeding. The range in the selection differentials for the single-plant lines is a result of the low survival of inbred material, the number of plants recorded in each progeny varying from 10 to 50. In the selfed lines, little effective selection was possible.

(ii) *Experimental layout*

In each year, seedlings from the original populations and from progenies of all generations were put out as spaced plants at 2 feet apart, together with clonal plants of the parents of all generations. Fifty seedlings of each progeny were used where possible. Smaller progenies, which occurred mainly in the single-plant lines, were not used unless 10 seedlings were available. In 1957 some 5000 seedlings and 1250 clonal plants were recorded.

TABLE 1

Coefficients of inbreeding and selection differentials under the mating systems employed

Mating system	Original population		First generation		Second generation		Third generation
	Coeff. of inbreeding	Selection differential	Coeff. of inbreeding	Selection differential	Coeff. of inbreeding	Selection differential	Coeff. of inbreeding
		Per cent.		Per cent.		Per cent.	
Fourway . .	0·0	3·2	0·0	4·0	0·125	8·0	0·219
Sib-crosses .	0·0	3·2	0·0	4·0	0·25	4·0	0·375
Sib-crosses/Fourway	0·0	3·2	0·0	4·0	0·25	4·0	0·25
Selfing/Sib-crosses	0·0	3·2	0·5	20-4	0·5	4·0	0·625
Selfing/Sib-crosses /Fourway	0·0	3·2	0·5	20-4	0·5	4·0	0·35
Selfed lines .	0·0	3·2	0·5	20-2	0·75	20-2	0·875

The results from clonal replication given earlier (Cooper, 1959*b*) suggested that block differences would not be significant, and that genotype/block interaction would be rather less than within-plant variation (developmental error). Consequently, in the experimental layout, the *progeny* seedlings were not replicated, but the clonal parents and the *population* seedlings were replicated in five blocks. As shown in table 8, no significant block differences could be detected, nor were there differences in within-plant variation between generations or between mating systems. The clonal replicates could therefore be used to give a satisfactory estimate of within-genotype variation.

3. RESULTS: MEAN RESPONSE TO SELECTION

In studying the results of any selection programme, two main effects are of interest ; firstly, the mean response to selection in relation to the selection differential applied, and secondly, the changes in genetic variation which occur within and between the lines.

A summary of the response to selection under the different mating systems in 1957 is given in table 3, while the behaviour of the fourway lines, and certain of the sib-mating lines, is shown in figs. 2 and 3. The results for 1956 in which only two generations were recorded are similar to those for 1957 and are not presented here. The environmental and within-plant errors for each generation or mating system can be obtained from table 8.

TABLE 2

Dates of ear emergence 1954-57; original populations and selected parents

		Reading 1954	Aberystwyth 1955	Gogerddan 1956	Gogerddan 1957
Irish					
Original population	Mean	3·4 May	14·0 May	8·7 May	30·4 Apr.
	Var.	24·0	30·7	12·4	18·7
	Range	23 Apr.-14 May	4-25 May	1-17 May	18 Apr.-18 May
Early parents . .	No. 1	23 Apr.	28·5 Apr.	1·2 May	24·6 Apr.
	2	24 Apr.	6·5 May	8·2 May	1·4 May
	3	24 Apr.	28·5 Apr.	2·8 May	24·6 Apr.
	4	25 Apr.	2·5 May	5·4 May	30·5 Apr.
Late parents . .	No. 1	14 May	26·5 May	21·4 May	16·0 May
	2	13 May	24·5 May	19·6 May	13·2 May
	3	14 May	24·0 May	16·0 May	6·8 May
	4	11 May	19·5 May	14·6 May	9·2 May
Kent					
Original population	Mean	24·1 May	29·7 May	22·5 May	22·8 May
	Var.	26·4	24·5	18·0	40·0
	Range	9 May-3 June	19 May-12 June	11-30 May	3 May-5 June
Early parents .	No. 1	13 May	22·0 May	18·4 May	12·2 May
	2	9 May	17·0 May	Dead	Dead
	3	11 May	15·0 May	15·8 May	10·8 May
	4	13 May	17·0 May	18·0 May	12·0 May
Late parents . .	No. 1	2 June	2·5 June	30·6 May	29·6 May
	2	2 June	3·5 June	8·2 June	3·4 June
	3	3 June	7·0 June	Dead	Dead
	4	2 June	3·5 June	31·2 May	2·0 June

(i) *Initial material*

The heading dates for 1954-57 of the original populations and of the initial parents are shown in table 2. There is a regular difference of three to four weeks in mean heading date between Irish and Kent ryegrass, but the latest plants of Irish overlap the earliest of Kent.

TABLE 3

Response to selection 1957

	Irish								Kent							
Original Population Mean . . . Var. . . . Range . . .	30·4 April 18·7 (18 A-18 M)								22·8 May 40·0 (3 M-5 J)							
	Early/early		Early/late		Late/early		Late/late		Early/early		Early/late		Late/early		Late/late	
Third generation lines A. Fourway crosses Mean . . . Var. . . . Range . .	20·8 A 21·9 (10-29 A)		9·9 M 29·7 (30 A-18 M)		6·4 M 15·8 (29 A-15 M)		13·0 M 18·8 (5-23 M)		28·2 A 24·2 (16 A-10 M)		22·7 M 12·3 (15-30 M)		25·4 M 15·7 (17 M-2 J)		8·7 J 10·0 (2-16 J)	
B. Sib-crosses/fourway Mean . . . Var. . . . Range . .	18.9 A 17.8 (10-23 A)		2.6 M 30·1 (23 A-16 M)		3·9 M 41·3 (24 A-12 M)		14.8 M 26·8 (8-23 M)		26·1 A 13·0 (17 A-2 M)		17·5 M 17·6 (11-28 M)		27·8 M 7·6 (24 M-2 J)		9·6 J 30·7 (30 M-21 J)	
C. Selfing/sib-crosses/ fourway Mean . . . Var. . . . Range . .	17·7 A 15·1 (10-23 A)		...		1·8 M 15·9 (27 A-13 M)		11·1 M 23·3 (4-25 M)		27·9 A 9·1 (20 A-3 M)		...		...		...	
D. Sib-crosses Mean . . . Var. . . . Range . .	(i) 19·2 A 38·6 8-30 A	(ii) 23·0 A 24·5 8 A-3 M	(i) 30·0 A 17·3 23 A-9 M	(ii) 8·9 M 27·6 2-19 M	(i) 2·3 M 12·1 27 A-11 M	(ii) 4·9 M 6·7 30 A-12 M	(i) 17·0 M 37·2 8-31 M	(ii) ...	(i) 1·2 M 40·8 23 A-24 M	(ii) 29·2 A 42·5 17 A-12 M	(i) 24·9 M 7·1 20-31 M	(ii) 18·5 M 9·3 13-23 M	(i) ...	(ii) 28·4 M 20·7 15 M-5 J	(i) ...	(ii) 17·5 J 14·3 11-21 J
E. Selfing once/sib-crosses Mean . . . Var. . . . Range . .	20·8 A 32·8 10 A-4 M	26·0 A 20·3 17 A-4 M	7·1 M 19·0 1-22 M	...	3·0 M 31·6 24 A-16 M	1·7 M 6·4 29 A-13 M	...	15·3 M 0·3 12-25 M	27·4 A 11·7 19 A-1 M	2·1 M 11·7 24 A-9 M	...	18·5 M 12·4 13-27 M	...	...	...	...
F. Continued selfing Mean . . . Var. . . . Range . .	...	...	6·5 M 14·5 30 A-13 M	8·0 M 10·3 4-11 M	29·6 A 16·3 23 A-8 M	...	...	...	1·4 M 9·5 27 A-10 M	8·8 M 70·4 24 A-30 M	...	...	...	...	...	...

... indicates that the line had died out by the third generation.

Kent usually shows a greater variance than Irish, and there is considerable variation between the early year 1957 and the late year 1955, this difference being greater for Irish than for Kent. A more

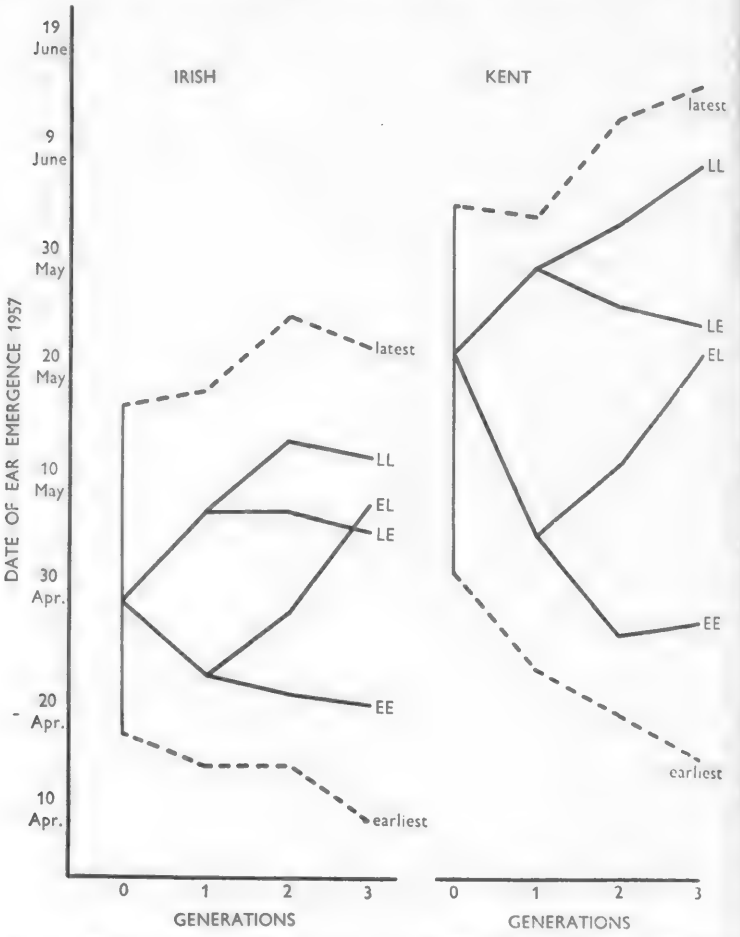

FIG. 2.—Response to selection for ear emergence in Irish and Kent ryegrass. Fourway crosses 1957.

——— Mean of selection lines

- - - - - Extreme early and late plants

detailed discussion of the heading behaviour of these strains has been presented earlier (Cooper, 1959*a*).

The parents selected as the earliest and latest in 1954 at Reading remained early and late respectively in subsequent years at Aberystwyth.

They did not, however, always represent the extreme range of the original population, particularly in the early year 1957, when in Irish ryegrass the clonal material of the original early parents was

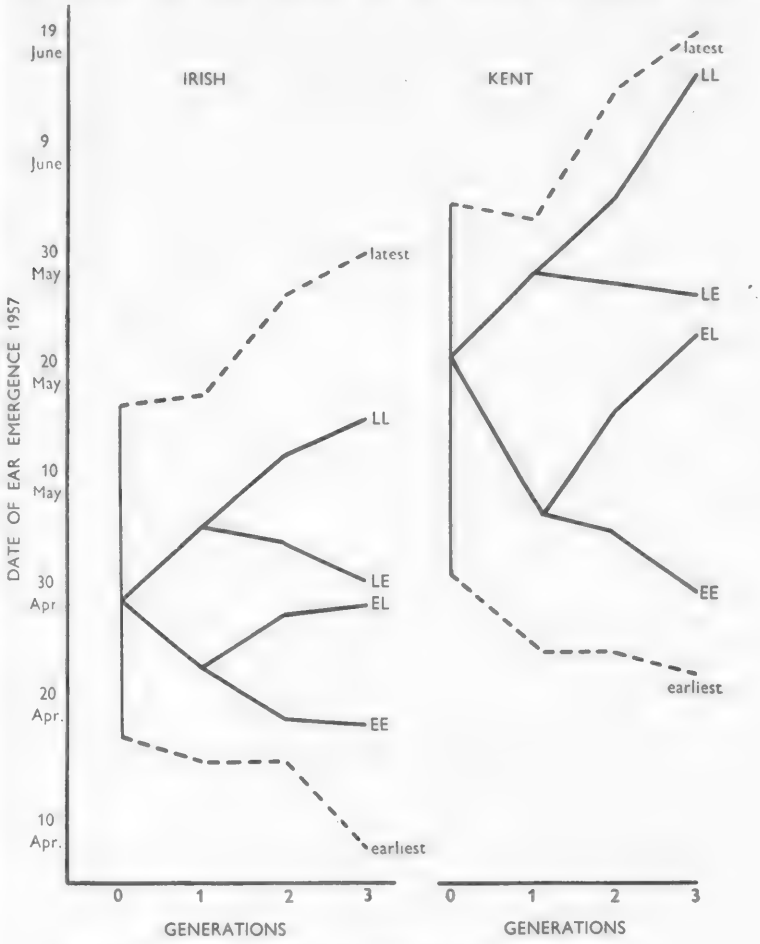

FIG. 3.—Response to selection for ear emergence in Irish and Kent ryegrass. Sib-crosses 1957.

——— Mean of selection lines

- - - - - Extreme early and late plants

some nine days later than the earliest of the population seedlings, and that of the late parents some six days earlier than the latest population seedlings.

(ii) *First generation*

The first generation of selection proved to be highly effective, as would be expected from the high additive genetic variation in the original populations (Cooper, 1959*b*). The progenies from selfing or pair-crossing, however, showed genetic variances of the same order as those of the original populations, indicating the heterozygous nature of the selected parents. The progenies from selfing showed marked inbreeding depression, being about five days later in both Irish and Kent, and this obscured the response towards earliness in the single-plant lines. As would be expected from the overlap of their parental distributions, the Irish late and Kent early groups were of similar heading date, and similar variance. The means of the early and late selection lines lay within the range of the original populations, but the extreme early and late plants were well outside. The same pattern of response was noted in all the three years, 1955-57, although in the cold spring of 1956, all dates of heading were later and the range between early and late material was reduced, compared to the warm spring of 1957.

(iii) *Second and third generations*

In these subsequent cycles of selection, the genetic variation available was that derived from a small group of plants of similar phenotype, and the response would not necessarily be as great as in the first generation. However, all the selected parents appear to be very heterozygous, and response continued during these two generations, although at a reduced rate in some lines. By the third generation, the extreme plants from both Irish and Kent were well outside the range of the original populations, and in Kent the *means* of the extreme EE and LL lines exceeded the original population range. Similarly, the late lines from Irish covered much of the range of the original Kent, and the early lines from Kent most of the range of the original Irish. (By the fourth generation, recorded in 1958, the late lines from Irish cover the full range of Kent ryegrass, and the early lines from Kent cover the full range of Irish.) There is no sign that the limits of selection have been reached, but this would not be expected by only the third generation. (The apparent lack of response in the LE and LL fourway lines in Irish does not continue in the fourth generation.) Evidently the genetic variation carried within even one individual is not yet exhausted.

The rate of response is not, however, the same for all groups and for all mating systems, and more detailed information on the efficacy of selection is presented in tables 4 and 5.

Table 4 shows the effect of each particular parental group on the response to selection, the response being measured by the difference between the means of the early and late lines from within each original parental group ; only the fourway crosses and the sib-mating/fourway outcrosses are included in this table.

This divergence is greater in the lines derived from early than from late parents, and is also greater in Kent than in Irish. This is particularly noticeable when the response from within the four late Irish plants is compared with that from the four early Kent plants. These initial groups have roughly the same heading date, but the early Kent

TABLE 4

Influence of parental groups on mean response to selection. Difference between means in third generation, 1957

	Irish			Kent			Kent-Irish	
	E/L-E/E	L/L-L/E	Difference	E/L-E/E	L/L-L/E	Difference	Early	Late
Fourway	19·1	6·6	12·5 (±1·31)	24·5	14·3	10·2 (±1·12)	5·4 (±1·34)	7·7 (±1·10)
Sib-crosses/ Fourway	13·7	10·9	2·8 (±1·52)	21·4	12·8	8·6 (±1·17)	7·7 (±1·25)	1·9 (±1·46)

TABLE 5

Differential response to selection for early and late ear emergence 1957

	Early/early lines			Early/late lines			Late/early lines			Late/late lines		
	Selection Differential (days)		Response (days)	Selection Differential (days)		Response (days)	Selection Differential (days)		Response (days)	Selection Differential (days)		Response (days)
	a	*b*		*a*	*b*		*a*	*b*		*a*	*b*	
Irish (family means)												
Fourway crosses .	1·6	14·8	3·5	18·0	17·8	13·0	2·0	10·9	3·0	19·7	8·0	5·8
Sib-crosses . .	1·1	16·6	2·2	16·7	16·1	11·2	3·0	10·4	4·5	21·0	7·9	9·9
Kent (family means)												
Fourway crosses .	5·1	13·8	8·7	22·0	10·9	14·2	−0·5	10·9	5·0	16·0	18·2	9·8
Sib-crosses . .	7·0	14·8	5·6	20·5	8·5	15·8	−3·3	14·1	2·0	22·2	12·8	18·1

a = calculated from clones and progenies in 1957.
b = calculated from parental records in 1955 and 1956.

group gave more than twice the response than did the late Irish. Two reasons for this difference may be suggested. Firstly, Kent ryegrass has been less stringently selected for uniformity of ear emergence than has Irish (Cooper, 1959*a*). Secondly, while late heads of an early-flowering genotype might be included in the seed crop of the late-flowering Kent, no such introgression is possible in an early-flowering strain.

More detailed information is provided in table 5 in which the responses to selection in the two directions were recorded separately and measured as the difference between the means of the first and third generation progenies. The cumulative selection differentials for these two generations have been expressed in two ways : (i) as the actual divergence in 1955 and 1956 between the family means and the parents selected from them, and (ii) as the difference between these same family means and clonal material of the selected parents when all were recorded in 1957. In this way it was hoped to assess the effect of genotype/year interaction on the selection differential.

Three important points arise from this table : (*a*) the response to selection is asymmetrical in all groups, the advance towards lateness being greater than that towards earliness, although the original selection differential was of the same order in both directions, (*b*) the response is of the same order in all the mating systems mentioned, there being no regular difference between the lines derived from four plants or from two plants, (*c*) there is a striking contrast between the *actual* cumulative selection differentials applied in 1955 and 1956, and the differences observed in 1957 between the corresponding family means and the clonal material of the parents selected from them. This is particularly noticeable in the lines selected for earliness, whether from early or late parental groups. In these lines, both the selection differentials and the observed responses as recorded in 1957 are comparatively small, but the cumulative selection differentials calculated from 1955 and 1956 data are far higher. The realised heritabilities therefore are high when calculated from the 1957 records but low when based on the actual selection differentials applied during the experiment. In the late lines, the agreement between the two measures of selection differential is better and the realised heritabilities are closer to the expected figures of 0·7-0·9 derived from parent/progeny regression within a single year (Cooper, 1959*b*).

These differences in selection differential from year to year may be due to genotype/year interaction. Selection for early heading can involve either a lower critical photoperiod, or the ability to grow at lower temperatures, and the particular mechanism selected may depend on the spring temperatures in the year of selection. It may be relevant to the present results that 1957 was a very warm spring, whereas in 1955 and 1956 the spring was cold. (Both the asymmetry in response, and the greater release of variation from Kent than from Irish, are confirmed in the fourth generation of selection in 1958.)

(iv) *Effect of mating system*

Up to the third generation, there has been no regular difference in the extent of response between the lines derived from one, two or four parents. The genetic variance within the lines derived from even one individual remains high, and the potential genetic variation within the initial groups is far from exhausted. Outcrossing in the

third generation (table 3) did not greatly influence the extent of response although in most cases the mean of the outcrossed sib-lines was earlier than that of the corresponding sib-crosses. Only enough single plant lines survived to produce four of the eight potential outcrosses, but here again the outcross means were all earlier than those of the corresponding sib-crosses. This increase in earliness may be a result of relaxation of inbreeding.

The most obvious effects of the different mating systems have been those associated with survival. In the extreme case of continued selfing (table 3) only five lines of Irish and five of Kent survived to 1956, and only three of each strain to 1957. Even in the initial selfings followed by sib-mating, only nine out of the sixteen potential lines could be maintained to 1957 ; in the sib-mating three lines out of sixteen have disappeared. This failure to maintain the inbred material is due partly to self-sterility and partly to low vigour and poor survival of the seedlings.

TABLE 6

Release of variation from initial groups (range in days), 1957

Original population	Irish			Kent		
	30			33		
1st generation .	20·0 (2)	18·8 (4)	Not available	19·5 (2)	16·8 (4)	Not available
2nd generation .	26·0 (2)	25·5 (4)	,,	30·0 (2)	24·3 (3)	,,
3rd generation .	31·0 (2)	35·3 (3)	34·0 (2)	37·0 (2)	37·0 (3)	33·0 (1)
	Four-plt. groups	Two-plt. groups	One-plt. groups	Four-plt. groups	Two-plt. groups	One-plt. groups

(Figures in brackets indicate number of lines in each group.)

4. RESULTS: CHANGES IN GENETIC VARIATION

It now remains to look more closely at the changes in the pattern of genetic variation which occur under selection, in particular at the extent of release of variation from within the initial groups, and the maintenance of genetic variation within lines under the different mating systems.

(i) *Release of variation from within initial groups*

Changes in the *mean* performance of the lines have already been considered, but the *variance* is also important since it determines the range between the extreme early and late plants and thus the possible selection differential for further advance. Table 6 shows the mean range in 1957 for the original populations and for the lines derived

from, one, two and four initial parents ; in the single-plant lines data are only available for the last generation.

While the mean range of the first generation progenies is well below that of the original populations, by the third generation of selection the range between the extreme plants derived from even one individual is equal to, or exceeds, that of the original population. There is no difference in the third generation between the ranges derived from initial groups of one, two or four plants, nor between those from Irish and from Kent. Evidently, one plant can carry more potential variation than is expressed phenotypically in the original population.

(ii) *Maintenance of genetic variation within lines*

The genetic variance of the selection lines would be expected to decline because (i) selection for an extreme phenotype might select

TABLE 7

Mean variances within selection lines, 1957

	Irish				Kent			
Original population	18·7				40·0			
1st generation	19·3 (2)	19·3 (4)	Not available	Not available	13·8 (2)	13·7 (4)	Not available	Not available
2nd generation	20·3 (4)	16·6 (8)	15·7 (2)	,,	16·1 (4)	20·1 (8)	31·6 (4)	,,
3rd generation	21·6 (4)	23·4 (7)	19·9 (6)	13·7 (3)	15·6 (4)	22·5 (7)	11·9 (3)	41·1 (3)
	Fourway	Sib-crosses	Selfing/sib-crosses	Selfed lines	Fourway	Sib-crosses	Selfing/sib-crosses	Selfed lines

(Figures in brackets indicate the number of lines in the group.)

homozygotes for plus or minus alleles, and (ii) inbreeding would tend to bring about homozygosis. The phenotypic variances of the original populations and the mean variances of the lines derived from them under the different mating systems are given in table 7.

In Irish ryegrass there is no regular decrease in variance accompanying selection and/or inbreeding, except possibly for the third generation selfed lines. With this exception, the phenotypic variances in the third generation are equal to, or greater than, that of the original population. In Kent, the variance of the original population is higher than in Irish, and also than in most of the selection lines. There is again no regular decrease in variance from the first to the third generation, and in Kent the mean variance of the selfed lines in the third generation is greater than that of the original population. It is evident

that even under sib-mating or selfing, considerable phenotypic variation is maintained in these lines over the first three generations. The next section shows that there is no regular change in the environmental or within-plant variation during these generations of selection, and one may conclude that these figures for a high phenotypic variance reflect the maintenance of a high genetic variation also within the selection lines.

(iii) *Developmental stability of inbred and outbred material*

In many outbreeding organisms, inbred or homozygous material is less well buffered against environmental changes and shows a lower developmental stability than the heterozygotes (Lerner, 1954). In the present material the developmental error (within-plant variation) was calculated from replicated clones for the original population plants and for the parents of the first two generations of selection. Block differences were also calculated separately for the different mating systems to see whether the inbred material was more sensitive to environmental fluctuations between blocks.

As can be seen from table 8, there is no obvious change in the developmental error of clonal material after two generations of selfing, or of selfing followed by sib-mating, nor is there any regular change in the variance between blocks following inbreeding. The present mating systems have not led to any decrease in the developmental stability of the selection lines.

5. DISCUSSION

The present paper deals with the first three generations of selection only, and the results cannot be compared with those of long-term response to selection, which have been reviewed by Mather (1953*a*, 1955), Lerner (1950), and Robertson (1955). It will be sufficient at this stage to compare the genetic structure revealed in these strains of *Lolium* with that of other outbreeding species, such as *Drosophila melanogaster*, and also to discuss the plant breeding implications of such a genetic structure.

(i) *Population structure*

One of the most interesting findings of the present work is the great potential variation which is carried within single individuals of *Lolium perenne* and capable of being released in a small number of generations. Similar storage of genetic variation has been reported in most outbreeding species which have been studied in detail (Mather, 1953*a*, Falconer, 1955; Manning, 1955) and considerable response to selection is usually possible.

In the present work, however, response has not followed a symmetrical pattern, the advance towards lateness being rather greater than that towards earliness. The most obvious immediate cause of

the asymmetry is the unequal selection differential in the two directions (table 5), but the reasons for this are obscure and need further study. Scaling effects can be ruled out, since the variance is not related to the mean, and the environmental switch, photoperiod, varies linearly over most of the response range. Inbreeding depression, however, results in later heading, and selection for early heading might therefore favour heterozygotes. The early lines might thus show less response than the late ones.

TABLE 8

Effect of mating system on developmental error of replicated clones, 1957

	Irish				Kent			
	Mean ear emergence	vError	vBlock	No. of clones	Mean ear emergence	vError	vBlock	No. of clones
Original population	1·9 M	4·25	0·12	38	24·9 M	2·93	−0·04	34
1st generation								
Outcrosses	6·9 M	4·58	0·86	8	23·6 M	3·12	0·08	15
Selfings	4·5 M	3·31	0·25	9	2·6 J	3·09	0·03	5
2nd generation								
Fourway crosses	9·5 M	3·99	1·77	12	21·8 M	2·08	−0·02	15
Sib-crosses	6·8 M	2·68	0·25	14	24·1 M	1·73	0·49	15
Selfings/sib-crosses	4·6 M	2·71	0·37	10	4·9 J	2·94	−0·38	5
Selfed lines	6·3 M	3·80	0·52	6	17·2 M	4·37	−0·49	6

Specimen analysis of variance

	dF	SS	MS	Expectation
Genotypes	37	5352·26	144·68	$V_E+5\,V_G$
Blocks	4	35·60	8·90	$V_E+38\,V_B$
Error	148	628·80	4·25	V_E
Total	189	6016·66	...	...

Hence $V_E = 4{\cdot}25$ $V_B = 0{\cdot}12$ $V_G = 28{\cdot}09$.

No selection limits have yet been reached, and would not be expected as early as the third generation. In some lines, however, both male and female fertility are decreasing, as found also by Mather and Harrison (1949) following selection for high and low chaeta number in *D. melanogaster*. This is more marked in the fourth generation, in which the parents selected for two lines (KLE and KEL) gave only nine and thirty-two seeds respectively and had to be replaced by less extreme plants.

In spite of the marked response to selection accompanied by inbreeding there has been little, if any, decrease in genetic variation

within these lines up to the third generation of selection, even in the closely inbred material. Several previous cases have been reported where selection or inbreeding have not led to the expected decline in genetic variation (Mather and Harrison, 1949 ; Lerner and Dempster, 1951 ; Manning, 1955) and this has usually been interpreted as due to natural selection against unbalanced gene combinations. Similar natural selection can be seen in the present work where most of the selfed lines died out, and there was also some mortality in the lines derived from selfing followed by sib-mating. This was due partly to self-sterility and partly low to vigour of the resulting seedlings, and selection for balanced heterozygotes might occur at both these stages. Such selection could also account for the absence of any increase in the developmental error of the inbred material.

Another possible mechanism for the maintenance of genetic variation is that of genotype/year or genotype/location interaction (Sprague, 1955 ; Robinson and Comstock, 1955). In field crops of ryegrass, for instance, the date of harvesting may vary with season or district, and thus affect the selective value of a particular heading date, while in the present experiment (tables 2 and 5) the heading dates of particular genotypes are affected differentially by yearly fluctuations in spring temperature.

The phenotypic variation within the selection lines will be influenced also by any changes in the developmental or environmental variation of the material during the progress of selection. The superior developmental stability (or homeostasis) of heterozygotes in outbreeding populations has been emphasised by Lerner (1954) but later studies suggest that the stability is not due to heterozygosity *per se*, but is under genetic control and is developed in the population under the action of selection (Mather, 1953*b* ; Jinks and Mather, 1955). In the present work, the inbred material shows no lower developmental stability for ear emergence than the outbred stocks, as measured either by the within-plant variance from replicated clones or by differences between blocks. The developmental stability of other ear characters (to be presented in a later paper) also shows little difference between the inbred and outbred material. It must be pointed out, however, that the plants surviving in the inbred material may well have been balanced heterozygotes selected for vigour.

One may conclude that the present populations of *L. perenne* show a similar genetic structure to that of other outbreeding organisms which have been studied in detail, in that most individuals are highly heterozygous and carry much potential genetic variation, which can be released quite rapidly for selection to act upon.

(ii) *Plant breeding implications*

The genetic structure of these outbreeding populations involving extreme heterozygosity of parents with considerable storage of genetic variation, contrasts with that of the self-fertilising cereals, in which

nearly all plants are homozygous, and most of the genetic variation is apparent in the phenotype. Many plant breeding concepts have been based on the situation in the cereals, and it is important to realise the difference in methods and outlook imposed by an outbreeding system.

Most of the genetic variation in a local population, or a bred strain, is hidden and it is selection rather than the potential content of the gene pool which determines the phenotypic range. It is sometimes thought that where a particular phenotype does not occur in an established strain, plant introduction or hybridisation is necessary to obtain it. While this may be true for certain major gene differences, such as disease resistance, it does not necessarily hold for polygenic characters. In the present experiment, for instance, by the fourth generation of selection for date of ear emergence, the full range of Irish for this character has been obtained from Kent, and that of Kent from Irish.

Pilot studies on such components of yield as leaf size, rate of leaf production and tillering have revealed a similar genetic structure for these characters, with considerable genetic variation within strains, and the response to selection for these characters is now under study. These findings suggest that, in a region such as Britain, where adapted forage species are already available, further improvement may be most usefully obtained by critical selection within adapted strains, rather than by the wide collection and introduction of new and unadapted ecotypes.

The genetic structure of the population also determines the optimum number of unrelated plants on which to base a strain. The difficulty is that too few basic plants may lead to inbreeding depression and loss of vigour in later generations of multiplication, while too many could result in excessive release of variation during multiplication.

In perennial ryegrass, Corkhill (1956) found that six parents were adequate, providing they showed high combining ability, while, earlier, Jenkin (1931) had suggested that eight unrelated plants were quite sufficient. The present results show that much genetic variation can be carried even within one individual, and that over the first three generations there is little difference in the rate of release from one, two or four parents. The results for ear characters (to be presented later) also indicate that for Irish and Kent ryegrass the fourway crosses are sufficient to maintain vigour for at least three generations of selection. These lines were continued using only four parents per generation, while in seed multiplication the full natural rate of increase is allowed. It seems likely therefore that four plants of similar phenotype would be quite adequate as the basis of a herbage grass strain.

Finally, the population structure influences the techniques of seed multiplication. In perennial ryegrass, even a carefully selected strain, based on a few parents of similar phenotype, will contain considerable potential genetic variation, and any selective action of seed multiplication can therefore result in genetic shift.

The problem of change in seed stocks during multiplication in regions other than the district of origin has been reviewed by Beard and Hollowell (1952). In the U.S.A., greater seed yields are obtained in the dry south-western states, such as Arizona and California, than in the humid north-east, but considerable changes in performance were reported in bluestems, blue grama, side-oats grama, switchgrass and brome-grasses when grown 300-400 miles north of the seed-producing areas.

Even where differences in climate are small, the selective effects of management, including such factors as the latest date of grazing in the spring, or date of cutting the seed-crop, may be important. In the late-flowering S.23 perennial ryegrass, for instance, a shift towards earliness of ear emergence during seed multiplication has been reported by Griffiths (1951), while Davies (1954) in this strain found an average change in mean heading date of 11·3 days between first and sixth generation stocks. More detailed figures for particular generations of multiplication have been given by Cooper (1959*c*) who found that up to five generations of multiplication could occur with only a slight shift in the date of heading. No corresponding changes in winter requirement before heading, or in leaf size, rate of leaf appearance or tillering in the seedling stage could be detected, although ample additive genetic variation for these characters exists in the strain.

It is evident that the mere existence of genetic variation in a strain does not necessarily lead to changes during seed multiplication. Whether these occur will depend on the direction and intensity of selection associated with the techniques of multiplication. In out-breeding species, such as perennial ryegrass, it is not possible to eliminate genetic variation in the initial stocks, and seed multiplication schemes are designed to minimise any changes between the foundation stocks and the certified seed as received by the farmer. Such schemes in the herbage grasses usually involve clonal propagation of foundation parents, and limitation of the number of generations of multiplication. An important addition to this list might be the use of a system of management which maintains the strain within the required phenotypic range.

6. SUMMARY

1. This paper describes the response to selection for date of ear emergence in Irish and Kent perennial ryegrass during the first three generations. The experiment was designed to compare the effects of different mating systems and different initial population sizes, and also to assess the genetic variation carried within small groups of parents.

2. Response has continued up to the third generation of selection in all lines and there is little difference in the rate of advance between the lines derived from one, two or four initial parents. Kent shows more rapid response than Irish, and in both strains the early parental

groups respond more rapidly than the late. In all cases, advance is asymmetrical, the shift towards lateness being greater than that towards earliness. By the fourth generation, the full range of Irish has been obtained from within Kent, and that of Kent from within Irish.

3. After three generations of selection, the range between the extreme early and late plants derived from a single individual is greater than that of the original population. One plant can evidently carry more potential genetic variation than is expressed phenotypically in the initial population.

4. Considerable genetic variation is maintained within the selection lines even with close inbreeding, and there is no increase in the developmental error within genotypes, nor in the fluctuations between blocks, following inbreeding.

5. It is concluded that *Lolium perenne* shows a similar genetic structure to that of other outbreeding organisms which have been studied in detail, such as *Drosophila melanogaster* and the mouse, in that most individuals are highly heterozygous and carry much potential genetic variation. The comparative uniformity of phenotype results from the limiting action of selection rather than from the absence of genetic variation.

6. The plant breeding implications of such a genetic structure are discussed, with particular reference to the optimum number of parents as the basis of a strain, and the problem of genetic shift during seed multiplication.

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THE GENETICS OF THE COLOUR PHASES OF THE ARCTIC SKUA

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1. INTRODUCTION

THE Arctic skua, *Stercorarius parasiticus*, is a colonial sea bird whose range extends from Scotland into the Arctic regions. It is of genetic interest due to the cline in colour phases which it shows. These phases appear to fall into three more or less well defined types—*pale*, *intermediate* and *dark*—with pale predominating in the North of the range and dark in the South. At the time, however, when a survey of this cline was made (Southern, 1943), intermediates were not recognised and it was not until Williamson's work on the breeding biology at Fair Isle (*e.g.* Williamson, 1951) that the existence of this third class was recognised. At Fair Isle, the breeding adults are trapped and colour ringed ; and the chicks—normally two in a brood—are caught and classified just before flying.

2. DESCRIPTION OF THE PHASES

The characters by which the phases may be recognised differ, of course, in the adults and chicks ; and, indeed, it will later be seen that the classification of chicks does not appear to correspond exactly with that of the adults.

In the adults, the pale phase is sharply marked off from intermediate ; but all graduations appear to exist between intermediate and dark. Pale is characterised by a white neck and belly ; in some birds the whole of the front of the body from the head downwards is white ; in others the white area on the neck is separated from that of the belly by a darker band. The back and wings of the bird are always dark. Occasionally a pale phase bird appears in which the white is replaced by a brownish white. This form has been called " dusky pale " at Fair Isle ; but it appears genetically identical to the other pales : the mating pale × dusky pale has always produced apparently normal pale phase chicks. Dark phases are distinguished by having head, neck and body of the same dark brown colour. The intermediates, however, show a wide range of variation: the most clearly marked individuals have white necks (the belly here always being dark), but there are others in which the " cheeks " are only slightly lighter than the rest of the body giving the head a " capped " appearance. These birds are called " dark-intermediates " at Fair Isle. But it is clear that all grades between intermediate and dark are present and the dividing line is arbitrary.

In the chicks the distinctions observed are less sharply marked between all phases than in the adults. Chicks which have been called pale show broad rufous tips to the feathers of the wings and back ; the belly feathers, especially those between the legs, are white, though they may be more or less tipped with brown. Intermediates generally have fewer and narrower tips to the feathers of the back and wings ; the belly feathers are white at the base but have a dark band of greater or

lesser extent at the tip. Dark chicks normally have completely dark feathers on the wings and back and on the belly, but some feathers may have narrow rufous tips : in any case it is the absence of white at the base of the feather that is judged to be the criterion. In general the belly characters seem to be more constant than the wings and back ; but there are naturally individuals in which classification into one or other form is arbitrary. It should be noted, however, that the classification of pale chicks appears to correspond with that of pale adults for pale × pale matings have always produced pale chicks. For the other matings, however, of thirteen chicks that have been recaptured as breeding adults, one was misclassified : a " dark-intermediate " chick became a dark adult. It is also interesting that an adult that was classified as " dark-intermediate " in one year became " dark " in the next. Clearly there is a problem of manifestation here.

3. THE BREEDING DATA

The matings pale × pale, pale × intermediate, pale × dark, intermediate × intermediate, intermediate × dark and dark × dark have been observed at Fair Isle since 1951 when Williamson first started the classification of intermediates. The colony has greatly increased in size, from twenty-five pairs in 1951 to sixty pairs in 1958. Table 1 shows the results obtained.

TABLE 1

Mating type	Chicks		
	P.	I.	D.
P × P . . .	29	0	0
P × I . . .	36	42	3
P × D . . .	16	39	2
I × I . . .	16	37	11
I × D . . .	9	125	43
D × D . . .	0	12	15

If it is assumed that two allelic genes are responsible for the existence of the three phases then clearly a certain proportion of the heterozygotes is liable to be misclassified as homozygous dark. It has already been mentioned that this is probable. It appears, however, that although the proportion of unrecognised heterozygotes among adults must be quite high, misclassification among the chicks can only occur in very slight degree. This is shown by the pale × intermediate matings where there can be no misclassification of adults : only three dark chicks have been produced. Similarly, among pale × dark matings, there are only two dark chicks. It should further be noted that if misclassification of chicks were at all serious, then in intermediate × intermediate matings an excess of darks should be observed : in fact the number of darks is below expectation. It is quite possible, the boundary between dark and intermediate chicks being indistinct, that there is two-way misclassification. For these reasons, we shall consider that the few darks thrown by the pale ×

intermediate and pale × dark matings are really intermediates and shall add them to the number of intermediates.

Suppose a proportion " λ " of the dark birds are really carrying the pale gene, *i.e.* they are heterozygous ; if we call the pale gene " p " and the dark gene " P ", then we have the following expectations for the three mating types (pale × dark, intermediate × dark and dark × dark) which supply information on " λ ".

A P × D mating will be $pp \times Pp$ with probability λ and $pp \times PP$ with probability $(1-\lambda)$, thus an offspring is phenotypically P with probability $\lambda/2$ and I with probability $\lambda/2+(1-\lambda)$, *i.e.* $1-\lambda/2$.

An I × D mating will be $Pp \times Pp$ with probability λ and $Pp \times PP$ with probability $(1-\lambda)$ thus an offspring is phenotypically P, I or D with respective probabilities $\lambda/4$, $\lambda/2+(1-\lambda)/2$, and $\lambda/4+(1-\lambda)/2$, *i.e.* $\lambda/4$, $1/2$ and $(2-\lambda)/4$.

A D × D mating is $Pp \times Pp$, $Pp \times PP$ or $PP \times PP$ with respective probabilities λ^2, $2\lambda(1-\lambda)$ and $(1-\lambda)^2$ from which it results that an offspring is phenotypically P, I or D with respective probabilities $\lambda^2/4$, $\lambda(2-\lambda)/2$, $(2-\lambda)^2/4$.

TABLE 2

Mating	Score, S	Information, I
P × D . .	$-16/\lambda+41/(2-\lambda)$	$57/\lambda(2-\lambda)$
I × D . .	$-9/\lambda+43/(2-\lambda)$	$177/2\lambda(2-\lambda)$
D × D . .	$-12/\lambda+42/(2-\lambda)$	$27+27(2-\lambda)/2\lambda+27\lambda/2(2-\lambda)$

Setting up the logarithmic likelihood expressions and differentiating with respect to λ we get the following formulæ shown in table 2 for the score S and information I of each of the three mating types P × D, I × D and D × D. By equating the sum of the scores to zero we obtain the estimate of λ based on the combined data. This is 0·453988.

The test of heterogeneity of the mating types in respect of the estimates of λ to which they separately lead is based on S^2/I (calculated for the value 0·453988 taken for λ), which is distributed as χ^2_2 (chi-squared for two degrees of freedom). This value comes out as 1·4502 (*i.e.* Probability 0·49). The agreement is clearly very good.

On this hypothesis we further expect the following ratios : P × I should give P : I :: 1 : 1 ; I × I should give P : I : D :: 1 : 2 : 1 ; and I × D should give I : P+D :: 1 : 1.

We have shown in table 3 the observed segregations and χ^2 for testing goodness of fit. Clearly the last figures contradict the 1 : 1 hypothesis. There are several reasons that could be put forward for this. The most simple assumes a viability disturbance : a deficiency of dark birds. An attempt at fitting, however, led to a very much reduced value for " λ " and this value did not then fit the pale × dark data. There remain two possibilities : inheritance of the phases may

depend on more than one locus ; or the effect may be due in some way to the mode of collecting the data. To test an hypothesis based on the assumption that two loci are involved would necessitate the fitting of two more parameters concerned with the frequencies of the alleles at each locus ; the further assumption would have to be made that union of gametes is at random. A simple dosage relation might be postulated : " *aabb* " as pale, " *aAbb* " and " *aabB* " as intermediate and the rest as dark. Even so, in the intermediate × dark matings it would still appear that an excess of darks should be shown, whatever the frequencies of " A " and " B ". The other possibility—of a bias in the classification of the chicks—would be very difficult to detect : this contingency is, however, indicated in the record books at Fair Isle where occasionally surprise is shown at a particular mating producing a particular chick ; and there is the very indeterminate boundary between phases in the chicks. The analysis of this factor must wait until a more objective means of classification has been devised.

TABLE 3

Mating	Observed segregation	χ^2	Degrees of freedom	Probability
P×I . .	36P+45I	1·0000	1	0·32
I×I . .	15P+37I+8D	4·9000	2	0·099
I×D . .	1251+52(P+D)	30·1073	1	<0·001

Owing to the very homogeneous estimate of " λ " which the data reveal, however, and the absence of any other plausible hypothesis, it seems that the one locus theory is the best that can be put forward at the moment. It is rather unfortunate that the recapture of chicks which have started to breed does not seem to throw any light on the problem : except that the one factor theory is not rejected by these data.

4. EVOLUTIONARY PROBLEMS

The existence of a cline in the proportion of the different phases has already been mentioned. It is therefore interesting to see whether this is a stationary cline of the type discussed by Fisher (1950) or a moving cline (Fisher, 1937). The numbers of the different phases for each year since 1951 at Fair Isle are as shown in table 4. A contingency χ^2 for fourteen degrees of freedom gave 7·535 which indicates that there is no reason to suppose any change in the relative proportions of the phases from 1951 to 1958.

This result does not necessarily prove that the cline is in this case a stationary one ; the breeding life of the Arctic skua appears to be fairly long ; and as the colony has increased each year, it seems doubtful that an equilibrium of the type postulated by Fisher can have

yet been set up. On the other hand, it would seem likely that some of the new birds which have arrived in the colony each year have come from other Orkney and Shetland colonies, and so it is not improbable that the relative proportions of the phases in this part of the cline have in fact remained more or less the same.

It seems possible that this cline may demonstrate another feature of great theoretical interest : this concerns the dominance relations within the cline.

The theory of a cline has been studied by Fisher as already indicated and Haldane (1948). Haldane considered the apparently unrealistic case of complete dominance with a gene at a constant selective advantage on one side of a boundary and at a constant disadvantage on the other. It appears more likely that there is a selective gradient with the gene frequencies maintained in equilibrium by

TABLE 4

Year	Phase			Total
	Pale	Intermediate	Dark	
1951	8	22	20	50
1952	11	20	21	52
1953	17	24	15	56
1954	15	33	20	68
1955	22	38	28	88
1956	26	47	31	104
1957	27	53	32	112
1958	25	57	39	121
Total	151	294	206	651

migration and selection : this is the case that Fisher considered and he also gave the apparatus for fitting. Fisher suggested that true dominance might indicate the very different mechanism of balanced polymorphism. It is clear that the evolution of true dominance can only occur once the population has become isolated from the migration taking place within the cline : the introduction of alien gene complexes must continually break down the modifier balance. There would seem, however, to be the possibility of a cline in dominance with a gene tending to be recessive in areas where it is low in frequency and dominant where it is abundant. If, as in Fisher's theory, there is a selective gradient along the cline, then selection must also be acting on genes modifying the expression and penetrance of the observed character. Thus we can expect not only an equilibrium between migration and selection acting on the factor itself, but also a series of such equilibria determining the frequencies of all the modifiers. Whether this will result in a cline in the dominance relations between the two alleles will depend on the number of modifiers

and the intensity of the selective gradient. Fisher adopts a standard unit of length such that

$$a^3 = 4k/g$$

where " k " is the coefficient of diffusion, " g " the selective gradient and " a " the distance separating the line on which the gene frequency is 10·04 per cent. from the line of 50 per cent. Since the selective intensity acting on the modifiers will be much less than that acting on the gene itself, it follows that the wave length of the frequency of each individual modifier will be much longer. Thus although there is no theoretical necessity for a cline in dominance to exist, yet the discovery of such a cline would provide very convincing evidence of the power of the evolution of dominance in nature.

That this is a possibility in the Arctic skua is shown by the fact that at Fair Isle the intermediates are very much more like darks than pales ; and, indeed, they appear to overlap the darks : pale must therefore show a tendency towards recessiveness. This is as the theory demands, for it must be assumed that in the southern part of the range where pales are the least frequent of the classes there is selection against pale. The proof of this theory must wait upon an examination of the genetics of the phases in colonies where pales are more abundant. A partial confirmation of this idea, however, is indicated in Southern's paper. He mentioned that L. S. V. Venables, who collected data in the Faeroes, noticed there the occurrence of intermediates and was unable to classify birds as either pale or dark in about half a dozen cases. This suggests that pale may start to show a tendency towards dominance in areas not very far north of the Shetlands.

5. SUMMARY

1. The three colour phases shown by Arctic skuas are described ; breeding data are given ; and a possible explanation of the genetic mechanism is put forward.

2. It is suggested, on the basis of observations on the numbers of the different phases made at Fair Isle since 1951, that the cline in these phases is a stationary one.

3. The theoretical possibility of a cline in dominance relations is discussed and it is noted that the Arctic skua could prove to be an example of this.

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FACTORIAL ANALYSIS OF BALANCED FOUR- AND HIGHER-POINT LINKAGE TESTS

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1. INTRODUCTION

BODMER and Parsons (1959) and Bodmer (1959) have given a method for a comprehensive analysis of balanced multi-point linkage experiments using the techniques of factorial experimentation. The cases of two and three points were discussed in detail. In this paper the method is extended to four- and higher-point data. The problem of distinguishing between additive and multiplicative systems, considered by Bodmer (1959), is discussed in relation to four-point data in the house mouse published by Parsons (1958).

2. FOUR-POINT DATA

The data from a complete n-point backcross linkage test can, in general, be set out in a $2^{n-1} \times 2^{n-1}$ latin square whose elements are the observations for pairs of complementary genotypes. As for the three-point test, the columns correspond to the modes of gamete formation,

TABLE 1

Scheme for the analysis of variance of a four-point test

	D.F.
Latin square of sums	
Parental heterozygote (rows)	7
Recombination (columns)	7
Two-factor interactions } (diagonals)	6
Four-factor interaction }	1
Error	42
	63
Latin square of differences	
Viability × Parental heterozygote (rows)	7
Viability × Recombination (columns)	7
Main effects and three-factor interactions with the exception of that represented by the total of the differences square (diagonals)	7
Error	42
	63
One of the main effects or three-factor interactions (that which represents the total of the differences square)	1
Total	127

the rows to the parental heterozygotes and the diagonals to the 2^{n-1} possible pairs of complementary genotypes. Thus the total S.S. (sum of squares) for any complete backcross linkage test consists of

$\frac{1}{2}$[S.S. of sums of complementary genotypes]$+\frac{1}{2}$[S.S. of the differences of complementary genotypes]$+$ a S.S. for the total of the differences. The first two items represent S.S. for latin squares which can be partitioned in the normal way into components for rows, columns, diagonals and an error term, whose meanings are exactly analogous to the meanings of the corresponding components in the three-point test discussed by Bodmer and Parsons (1959).

For a four-point test the data may be set out in an 8×8 square with 128 classes. The scheme for the analysis of variance is given in table 1, and is identical with the three-point situation except for the allocation of viabilities and their interactions.

There are six two-factor interactions and one four-factor interaction. The S.S. for the two- and four-factor interactions is, symbolically,

$$\begin{aligned}&\tfrac{1}{128}[\{(a-1)(b-1)(c+1)(d+1)\}^2+\{(a-1)(b+1)(c-1)(d+1)\}^2\\&\quad+\{(a-1)(b+1)(c+1)(d-1)\}^2+\{(a+1)(b-1)(c-1)(d+1)\}^2\\&\quad+\{(a+1)(b-1)(c+1)(d-1)\}^2+\{(a+1)(b+1)(c-1)(d-1)\}^2\\&\quad+\{(a-1)(b-1)(c-1)(d-1)\}^2]\end{aligned}$$

$$= \tfrac{1}{16}\begin{bmatrix}(abcd+1)^2\\+(a+bcd)^2\\+(b+acd)^2\\+(c+abd)^2\\+(cd+ab)^2\\+(ad+bc)^2\\+(bd+ac)^2\\+(abc+d)^2\end{bmatrix} - \tfrac{1}{128}\begin{bmatrix}(abcd+1)\\+(a+bcd)\\+(b+acd)\\+(c+abd)\\+(cd+ab)\\+(ad+bc)\\+(bd+ac)\\+(abc+d)\end{bmatrix}^2$$

where *abcd*, etc. represent the total contributions of the corresponding genotypes to the eight modes of gamete formation. This is the diagonals S.S. of the sums square as shown in the analysis of variance.

The interpretation of the diagonals S.S. for the differences square is a little more difficult. The *abc* interaction may be written symbolically as,

$$\begin{aligned}&(a-1)(b-1)(c-1)(d+1)\\&\quad=(abcd-1)+(a-bcd)+(b-acd)+(c-abd)+(cd-ab)+(ad-bc)\\&\qquad+(bd-ac)+(abc-d)\end{aligned}$$

If the differences square is made up by taking the differences between complementary pairs as is shown in this expression, the S.S. for the total of the differences square will represent the *abc* interaction. The diagonals S.S. of the differences square can then be shown to represent the four main viability effects and three of the four three-factor interactions, the fourth being the *abc* interaction.

Thus the composition of the diagonals S.S. of the differences square depends on the interaction that is represented by the total of the differences square.

3. FIVE-POINT DATA

The data from a complete five-point test may be set out in a 16×16 square split into complementary pairs giving a total of 512 classes leaving 511 D.F. after the restriction that the total observed must equal the total expected. The 511 D.F. may be split as in table 2.

TABLE 2

Scheme for the analysis of variance of a five-point test

	D.F.
Latin square of sums	
Parental heterozygotes (rows)	15
Recombination (columns)	15
Two-factor interactions (diagonals)	10
Four-factor interactions (diagonals)	5
Error	210
	255
Latin square of differences	
Viability × Parental heterozygote (rows)	15
Viability × Recombination (columns)	15
Main effects (diagonals)	10
Three-factor interactions (diagonals)	5
Error	210
	255
Five-factor interaction	1
Total	511

In this case, the S.S. of the sum of the differences square is arranged to represent the five-factor interaction $(a-1)(b-1)(c-1)(d-1)(e-1)$, although it could equally well represent a main effect or three-factor interaction.

In the three-point situation discussed by Bodmer and Parsons (1959) the S.S. for the total of the differences square represents the *abc* interaction, although the calculation could be equally well done if the sum of the differences square represented one of the three main effects. In the two-point situation, the S.S. of the total of the differences square represents either the main effect of *a* or of *b*.

This analysis may be extended to data involving six or more factors, although it is unlikely that experiments of this magnitude would be undertaken.

4. CONFOUNDING IN FOUR- AND HIGHER-POINT DATA

It was pointed out by Bodmer and Parsons (1959), that, as in agricultural factorial experiments, if we are willing to neglect higher-order interactions, the technique of confounding may be used to reduce the number of heterozygotes needed in a balanced multi-point backcross linkage experiment.

Only interactions involving an even number of factors can be confounded, as the complementary genotypes cannot be separated.

The general problem of selecting such subsets is complicated, but a scheme for finding them has been devised by Edwards (1958). We shall consider in detail the analysis needed for a confounded four-point test.

Bodmer and Parsons (1959) showed how it was possible to confound the four-factor interaction in a four-point test and so reduce the number of heterozygotes needed from 8 to 4. The data from such a set of four heterozygotes can be set out as in table 3, where a genotype is designated by small letters for the loci at which it is homozygous. The four pairs of complementary genotypes (1, *abcd*), (*bc*, *ad*), (*cd*, *ab*) and (*bd*, *ac*) are arranged in a latin square whose rows correspond to

TABLE 3

Genotypes from the four heterozygotes which constitute a balanced set

Parental heterozygote	Modes of gamete formation							
	(0)	(2)	(123)	(13)	(1)	(12)	(23)	(3)
$\frac{a\ b\ c\ d}{+\ +\ +\ +}$	*abcd* 1	*cd* *ab*	*bd* *ac*	*ad* *bc*	*a* *bcd*	*b* *acd*	*c* *abd*	*d* *abc*
$\frac{a\ b\ +\ +}{+\ +\ c\ d}$	*cd* *ab*	*abcd* 1	*ad* *bc*	*bd* *ac*	*b* *acd*	*a* *bcd*	*d* *abc*	*c* *abd*
$\frac{+\ b\ +\ d}{a\ +\ c\ +}$	*bd* *ac*	*ad* *bc*	*abcd* 1	*cd* *ab*	*c* *abd*	*d* *abc*	*a* *bcd*	*b* *acd*
$\frac{+\ b\ c\ +}{a\ +\ +\ d}$	*ad* *bc*	*bd* *ac*	*cd* *ab*	*abcd* 1	*d* *abc*	*c* *abd*	*b* *acd*	*a* *bcd*

the heterozygotes, and columns to the four modes of gamete formation (0), (2), (13) and (123). The remaining pairs of genotypes form a second latin square whose columns are the modes of gamete formation (1), (12), (3) and (23). The total S.S. for data from such an experiment may therefore be split into the S.S. from these two latin squares, which we shall call A and B, say, and the S.S. for the difference between the totals of the squares. This latter is then the confounded degree of freedom. The S.S. for each of A and B may then be split up as in the analysis of the three-point experiment into a latin square of sums, a latin square of differences, and a contribution from the total of the differences square. The interpretation of the components is similar to that considered in previous sections, except that corresponding components from A and B have to be combined to make them represent the viability effects and interactions. Thus if the differences squares are arranged so that their total represents the *abc*-interaction

we have

$$8\times abc \text{ interaction} = (abcd-1)+(cd-ab)+(ad-bc)+(bd-ac)+(a-bcd)+(b-acd)+(abc-d)+(c-abd)$$
$$= y_A+y_B, \text{ say}$$
$$\text{and } 8\times d\text{-effect} = (abcd-1)+(cd-ab)+(ad-bc)+(bd-ac)-[(a-bcd)+(b-acd)+(abc-d)+(c-abd)]$$
$$= y_A-y_B$$

where y_A and y_B represent the totals of the differences squares from A and B. Hence the S.S. from the totals of the differences squares, which is

$$\tfrac{1}{32}(y^2{}_A+y^2{}_B) = \tfrac{1}{64}(y_A+y_B)^2+\tfrac{1}{64}(y_A-y_B)^2,$$

is also the S.S. for the *abc*-interaction and the *d*-effect. In a similar way it can be shown that the S.S. for the diagonals from the two differences squares is the S.S. for the remaining one- and three-factor effects, and the S.S. for the diagonals of the two sums squares is the S.S. for the two-factor interactions. The heterozygote effect will be represented by the S.S. of the totals of the heterozygote contributions, and when subtracted from the S.S. for the rows of the sums squares, leaves a residual representing interaction between viabilities and modes

TABLE 4

Scheme for the analysis of a confounded four-point experiment

	D.F.
Parental heterozygotes } from S.S. for rows of sums squares	3
Residual interaction }	3
Recombination—from S.S. for columns of sums squares	6
Main viability effects	4
Two-factor interactions	6
Three-factor interactions	4
Viability × Parental heterozygote—from S.S. for rows of differences squares	6
Viability × Recombination—from S.S. for columns of differences squares	6
E_1 } error terms from the four latin squares	6
E_2 }	6
E_3 }	6
E_4 }	6
Confounded degree of freedom—from S.S. for the difference between A and B	1
Total	63

of gamete formation. The S.S. for rows and columns of the differences squares have their usual interpretation as viability × heterozygote and viability × recombination interactions and there are four error terms. The resulting scheme for the complete analysis of a confounded four-point experiment is given in table 4.

5. NUMERICAL APPLICATIONS TO FOUR-POINT DATA

Data for a complete four-point backcross linkage test performed on the house mouse were given by Parsons (1958). They involve the factors *fuzzy* (*fz*), *Splotch* (*Sp*), *leaden* (*ln*) and *polydactyly* (*py*), of which

all except the second are recessive. Since we are interested in the effect of *Sp* on viability and this only occurs heterozygously, we must consider the heterozygote *Sp*+ as representing the same treatment levels as the recessive homozygotes *fzfz*, *lnln*, *pypy*. The analysis of χ^2 on the complete male data is presented in table 5, where the total of

TABLE 5

Analysis of χ^2 of Parsons' (1958) four-point male data for the house mouse

	D.F.	χ^2	P in per cent.
Latin square of sums			
Parental heterozygotes (rows)	7	255·12	$<<0{\cdot}1$
Recombination (columns)	7	3687·36	$<<<0{\cdot}1$
Two-factor and four-factor interactions (diagonals)	7	39·27	$<0{\cdot}1$
fz Sp-interaction	1	2·68	...
fz ln-interaction	1	0·28	...
Sp-ln-interaction	1	2·19	...
fz py-interaction	1	0·37	...
Sp py-interaction	1	8·86	0·5-0·1
ln py-interaction	1	23·71	$<0{\cdot}1$
fz ln Sp py-interaction	1	1·18	
Error	42	418·93	$<<0{\cdot}1$
	63		
Latin square of differences			
Viability × Parental heterozygote (rows)	7	5·34	...
Viability × Recombination (columns)	7	15·29	5·2·5
Main effects and three-factor interactions other than the *fz ln py*-interaction (diagonals)	7	32·94	$<0{\cdot}1$
Main effect of *fz*	1	13·59	$<0{\cdot}1$
Main effect of *Sp*	1	3·51	10-5
Main effect of *ln*	1	0·42	...
Main effect of *py*	1	13·88	$<0{\cdot}1$
fz Sp py-interaction	1	0·59	...
fz Sp ln-interaction	1	0·48	...
Sp ln py-interaction	1	1·01	...
Error	42	68·92	1-0·1
	63		
fz ln py-interaction (total of differences square)	1	0·21	...
	127	4523·38	

the differences square has been taken to represent the *fz ln py* interaction. Only significance levels less than 10 per cent. are indicated.

By far the largest component is that due to recombination. There is also very severe non-orthogonality indicated by the highly significant parental heterozygote component. Such severe non-orthogonality may seriously affect the terms for viability interactions involving an even number of factors and will in general make the χ^2 analysis somewhat inaccurate. This accounts for the significant error term in the sums square, and also for the apparent *Sp py* and *ln py* interactions.

Terms in the differences square are less affected by non-orthogonality, although even here the error term is significant as also is the viability × recombination interaction. This latter represents an effect which could have no simple biological meaning and its significance is almost certainly due to the non-orthogonality. Some confidence can be placed in the significant effects for *fz* and *py*. The factor *fz* is well known to have a somewhat deleterious effect. Polydactyly is not generally associated with viability deficiency, but was, in the stock used by Parsons, not perfectly manifesting. It was shown by Parsons (1958) that the polydactyly effect in these data was almost certainly due to misclassification of the polydactyly and not to any viability disturbance. The problem of estimating recombination in multiple linkage tests with one factor imperfectly manifesting has been considered by Parsons (1957). An examination of the scheme of expectations given for such a situation indicates that the general effect of misclassification on the analysis of χ^2 will be indistinguishable from the effect of a corresponding viability deficiency. The *Sp* effect is a little inflated which might be interpreted as an indication of a slight viability deficiency. It is, however, the largest of the remaining six interactions involving an odd number of factors, and so its significance must be modified accordingly. The three-factor interactions are all well below significance, as would be expected.

It has been pointed out that the differences square can be arranged in a number of ways, according to which effect will be represented by its total. It is clear that these will in general result in different values for the rows, columns and error terms of the differences square. Each arrangement will give legitimate measures for viability × heterozygote and viability × recombination interactions. However if the error term is significant, usually indicating either severe non-orthogonality, or disturbances not taken into account by the analysis, these components may differ considerably for different arrangements of the differences square, and thus not provide reliable measures of viability × recombination and viability × heterozygote interactions.

The data from the four heterozygotes

$$\frac{fz+ln\ py}{+Sp++},\ \frac{fz+++}{+Sp\ ln\ py},\ \frac{++++py}{fz\ Sp\ ln+}\ \text{and}\ \frac{++ln+}{fz\ Sp+py}$$

only, have been analysed as if they represented the outcome of a confounded four-point experiment. The resulting analysis of χ^2 is given in table 6, where as before, the total of the differences square represents the *fz ln py*-interaction. It is on the whole similar to that for the complete data. The effect of the non-orthogonality on the two-factor interactions is more severe, but the main effects and three-factor interactions are still not disturbed by the non-orthogonality. The significance of the residual interaction of 3 D.F. is also a result of the non-orthogonality. The most striking difference is the non-significance of the *py*-effect. This is, however, in the same direction as that for the

complete experiment and a heterogeneity χ_1^2 comparing the ratio of polydactyls to normals for the two halves of the experiment gives only 1·42.

It is clear that in order to obtain more reliable measures of the viability effects and interactions, some sort of logarithic transformation is needed. The logarithmic transformation cannot be applied to the data as they stand, because of the small numbers of observations in some of the classes. However, if we take the logarithms of the

TABLE 6

Analysis of a confounded four-point experiment based on Parsons' (1958) data

		D.F.	χ^2	P in per cent.
Parental heterozygotes		3	149·08	$<<0·1$
Residual interaction		3	11·05	≈ 1
Recombination		6	1591·14	$<<0·1$
Viability × heterozygote		6	5·45	...
Viability × recombination		6	14·21	5-2·5
Main effects	*fz*	1	7·24	1-0·5
	Sp	1	0·20	...
	ln	1	3·70	10-5
	py	1	2·46	...
Two-factor interactions	*fz Sp*	1	11·98	$<0·1$
	fz ln	1	3·26	10-5
	Sp ln	1	41·96	$<<0·1$
	fz py	1	3·26	10-5
	Sp py	1	9·65	0·5-0·1
	ln py	1	28·66	$<0·1$
Three-factor interactions	*fz Sp ln*	1	1·33	...
	fz Sp py	1	0·20	...
	fz ln py	1	0·64	...
	Sp ln py	1	1·62	...
E_1 } from sums squares		6	134·76	$<<0·1$
E_2 } from sums squares		6	31·93	$<0·1$
E_3 } from differences squares		6	12·36	≈ 5
E_4 } from differences squares		6	3·18	...
Confounded degree of freedom		1	3·05	10-5
		63		

totals observed for each genotype, we will at least obtain unbiased measures of multiplicative effects involving an odd number of factors. Thus in terms of logarithms the *fz*-effect, for example, is

$$\tfrac{1}{8}[\log fz+\log fz\ Sp+\log fz\ ln+\log fz\ py+\log fz\ Sp\ ln+\log fz\ Sp\ py+\log fz\ ln\ py+\log fz\ Sp\ ln\ py-\log Sp-\log ln-\log py-\log Sp\ ln-\log Sp\ py-\log ln\ py-\log Sp\ ln\ py-\log 1]$$

$$= \log_8 \sqrt{\frac{fz.fzSp.fzln.fzpy.fzSpln.fzSppy.fzlnpy.fzSplnpy}{Sp.ln.py.Spln.Sppy.lnpy.Splnpy.1}}$$

where *fz*, *Sp*, etc. and 1 represent the totals observed for the corresponding genotypes. The antilog. of this will be the multiplicative effect of *fz*. Using Fisher's (1925-54) approximate formula for the variance of a statistic expressed as a function of the observed frequencies,

it is easily seen that the variance of each of the logarithmic multiplicative effects is $\frac{1}{64} \times$ the sum of the reciprocals of the genotype totals. If logarithms are taken to the base ten, this must be multiplied by $(\log_e 10)^2$. The square of each effect divided by this variance provides an approximate χ^2_1 for measuring the significance of the effect. These χ^2_1's are shown in table 7 together with the multiplicative effects expressed as a percentage of standard. The results are very similar to those obtained by the analysis on the original data, and still show a

TABLE 7

Tests for multiplicative viability effects in Parsons' (1958) four-point data

	Multiplicative effects in per cent.	χ^2_1	P in per cent.
Main effects			
fz	85·63	15·01	<0·1
Sp	92·64	3·53	10-5
ln	103·1	0·59	...
py	86·86	12·41	<0·1
Two-factor interactions			
fz Sp	92·68	3·60	10-5
fz ln	97·31	0·44	...
Sp ln	93·11	3·18	10-5
fz py	102·4	0·35	...
Sp py	113·2	9·61	0·5-0·1
ln py	123·0	26·72	<0·1
Three-factor interactions			
fz Sp ln	96·56	0·76	...
fz Sp py	104·1	0·98	...
fz ln py	103·9	0·90	...
Sp ln py	104·8	1·37	...
Four-factor interaction			
fz Sp ln py	105·6	1·85	...

marked disturbance of the two-factor interactions by the non-orthogonality. The additive effect of *fz*, for example, was −2·9219 relative to an overall mean of 20·1016, and the corresponding multiplicative effect is −2·8886. The similarity between the two analyses shows that the effects are too small for a difference between additive and multiplicative systems to be detectable. The estimate for the fuzzy viability of 85·63 per cent. has a standard error of 3·43. An examination of the expectations given by Parsons (1957) for situations in which there is misclassification of one factor shows that if the *py*-effect is entirely due to misclassification it will approximately be an estimate of $(1-\lambda)/(1+\lambda)$ where λ is the percentage misclassification of *py*. This gives an estimate for λ of $7{\cdot}032 \pm 1{\cdot}992$ per cent. which is in close agreement with the value obtained by Parsons (1958) of $6{\cdot}910 \pm 1{\cdot}959$ per cent., by a different method.

As there is no real evidence for any interactions involving *Sp*, it is legitimate to neglect *Sp* and consider the results as if they had been obtained from a three-point experiment involving *fz*, *ln* and *py*. The data are then no longer too sparse for a direct application of the logarithmic transformation and so make possible an investigation of the effects affected by severe non-orthogonality in the analysis on the original data. The analysis of the data considered in this form and after taking logarithms is given in table 8. It should be noticed that

TABLE 8

Analysis, after taking logarithms, of Parsons' (1958) data considered as a three-point test involving fz, ln *and* py

	D.F.	χ^2	P in per cent.
Latin square of sums			
Parental heterozygotes (rows)	3	166·94	$<<0{\cdot}1$
Recombination (columns)	3	1483·87	$<<<0{\cdot}1$
Two-factor interactions (diagonals)	3	6·61	10-5
fz ln-interaction 1		3·79	≈5
fz py-interaction 1		0·09	...
ln py-interaction 1		2·73	≈10
Error	6	11·81	10-5
	15		
Latin square of differences			
Viability × parental heterozygote (rows)	3	4·48	...
Viability × recombination (columns)	3	3·96	...
Main effects (diagonals)	3	28·26	$<0{\cdot}1$
main effect of *fz* 1		15·69	$<0{\cdot}1$
main effect of *ln* 1		0·21	...
main effect of *py* 1		12·36	$<0{\cdot}1$
Error	6	8·65	...
	15		
fz ln py-interaction (total of differences square)	1	2·56	...
	31		

the effects tested are multiplicative effects as defined above. The error term of the sums square and two of the two-factor interactions are still slightly inflated by the non-orthogonality, but the differences square is no longer affected. There is clearly no viability × heterozygote or viability × recombination interaction. The power of the logarithmic transformation in representing the situation on a multiplicative basis and eliminating the effects of the non-orthogonality is quite striking.

6. DISCUSSION

The main purpose of this paper has been to apply the factorial analysis of balanced multi-point linkage tests developed for two- and three-point data to four- and higher-point data. The application to the situation where all the possible multiple heterozygotes are used

as parents in four- and higher-point data is a mere extension of the previous work. The only difficulty is the allocation of the viability effects and interactions.

However, the technique of confounding may be used to reduce the number of heterozygotes needed in four- and higher-point experiments. In a four-point experiment, therefore, by confounding the *abcd* or four-factor interaction it is possible to reduce the number of heterozygotes needed from eight to four. The analysis of variance for such a balanced set of four is discussed and provides information on all the components that the complete analysis gives with the exception of the confounded *abcd* interaction. The analysis of balanced sets formed by confounding various four and higher even-order interactions will be of greater importance in five- and higher-point data where it would be increasingly laborious to make up all the possible multiple heterozygotes.

The techniques developed in this paper are illustrated with an analysis of χ^2 of some four-point data in the house mouse given by Parsons (1958). The analysis gives a large χ^2 for the parental heterozygote component indicating non-orthogonality and this accounts for large two-factor interactions, viability $\times$ recombination interactions and error terms. In a situation where the error term of the " differences " square is large, the magnitude of the components of the " differences " square may vary considerably according to the viability interaction represented by the total of the differences square. Thus measures of viability $\times$ recombination and viability $\times$ heterozygote interactions will be unreliable under such circumstances.

However, after taking logarithms and analysing the resulting data on the basis of a multiplicative system, the significant interactions and error terms noted above were reduced on the whole to insignificance. Thus the power of the logarithmic transformation in representing the situation on a multiplicative basis is clearly demonstrated. Further evidence of the power of the logarithmic transformation is provided by data of Parsons (1959) in *Drosophila melanogaster* where many interactions are reduced to insignificance after taking logarithms.

7. SUMMARY

1. The factorial analysis of complete four- and five-point linkage data is discussed and the extension to higher-point data indicated.

2. The analysis of a balanced set in a four-point linkage test formed by confounding the four-factor interaction is presented.

3. Four-point data in the house mouse (Parsons, 1958) is analysed to illustrate the methods for both the complete data and the balanced set.

4. The data are analysed after taking logarithms as proposed by Bodmer (1959) and it is shown that the logarithmic transformation provides a more realistic picture of the situation. This supports the multiplicative basis of expectations assumed for such experiments.

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GENETICS OF MELIBIOSE FERMENTATION IN *SACCHAROMYCES ITALICUS* VAR. *MELIBIOSI*

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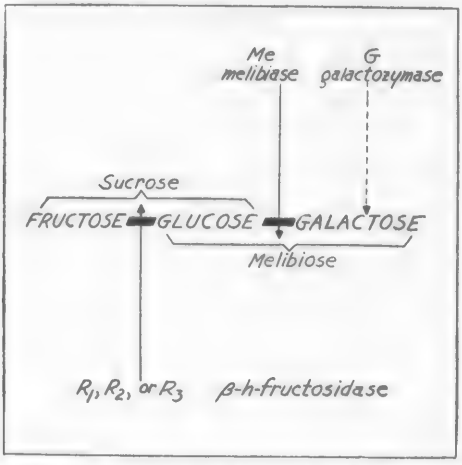
1. INTRODUCTION

THE problem of genic control of sugar fermentation by the yeasts has been investigated in considerable detail during the past twenty years. Lindegren, Spiegelman and Lindegren (1944) were the first to investigate the genetics of melibiose fermentation by producing hybrids between *S. carlsbergensis*, which ferments melibiose, and *S. cerevisiae*, which is a melibiose non-fermenter. Their tetrad analysis yielded a 2-gene segregation, and they concluded that *S. carlsbergensis* possesses two dominant genes for melibiose fermentation and *S. cerevisiae*, the recessive alleles. Another hybrid between the same two species was analysed by Spiegelman, Lindegren and Lindegren (1945) and 10 asci yielded only 2 : 2 segregation ratios, indicating that the parent strain *S. carlsbergensis* must have contained only one gene for melibiase synthesis. The authors also studied the adaptive nature of melibiose fermentation : when the cultures from the same 10 asci were maintained in the presence of melibiose, 6 of them were found to yield 4 : 0 ratios and then to revert to the original 2 : 2 ratios upon the removal of melibiose from the substratum. This phenomenon was explained on the basis of enzymatic synthesis and cytoplasmic transfer in the absence of the gene ; confirmation of these results has not been obtained. Winge and Roberts (1957) likewise studied melibiose fermentation, but because of the low fertility of their original hybrid, they were unable to determine whether *S. carlsbergensis* contains more than one Me-gene. On the other hand, Gilliland (1956), as a result of tetrad analyses of a hybrid between *S. carlsbergensis* and *S. cerevisiae* var. *ellipsoideus* concluded that only one melibiase gene was present.

Yeasts which possess one or more of the R-genes for the synthesis of β-h-fructosidase are able to ferment raffinose by splitting off the fructose portion of the molecule, which is in turn fermented by the zymase (hexokinase) system present in all fermenting yeasts ; the remaining melibiose portion of the molecule is left intact, providing that the melibiase gene is absent. In the presence of Me, the melibiose is split into galactose and glucose. The raffinose molecule can also be fermented by a completely different process in the absence of the R-gene. In the single spore progeny of the hybrid *S. carlsbergensis* $\times$ a recessive, Winge and Roberts (1957) found that those isolates which lacked the R-gene were nevertheless able to ferment raffinose 1/3 due to the complementary action of Me and G (galactozymase gene). The former acted on the galactosidic linkage, liberating galactose

and sucrose, while the G-gene brought about the customary fermentation of galactose. Thus, 1/3 raffinose fermentation results when either



TEXT-FIG. 1.—Gene action involved in the hydrolysis and fermentation of the raffinose molecule (after Winge and Roberts, 1957).

R' or Me+G is present, whereas when all three genes are present, as in *S. carlsbergensis*, the molecule is fermented completely (text-fig. 1).

TABLE 1

A comparison of the fermentative ability and genotype of the three different yeasts under discussion

Yeast	Fermentative ability	Genes present
S. carlsbergensis . . .	Raffinose+(1/1) Sucrose+ Melibiose+ Galactose+ Maltose+	R_2 (and perhaps others) R_2 Me (and perhaps others) G M_6 (and perhaps others)
S. italicus	Raffinose− Sucrose− Melibiose− Galactose+ Maltose+	r r me G M
S. italicus var. *melibiosi*	Raffinose+(1/3) Sucrose− Melibiose+ Galactose+ Maltose+	r r Me (?) G (?) M (?)

This demonstration of the role played by complementary genes in the fermentation of raffinose was based on a genetic investigation and was fully confirmed by the biochemical study of Losada (1957), who demonstrated that the melibiase found in *S. carlsbergensis* is capable of hydrolysing the raffinose molecule. A similar conclusion with regard to *S. italicus* was independently reached by van Uden and Assis-Lopes (1957) who worked with strains isolated from the digestive tract of both man and horse. These strains differed from the standard description of the species (Lodder and Kreger-van Rij, 1952) in being able to ferment melibiose and raffinose 1/3 and were therefore relegated to a new variety, *S. italicus* var. *melibiosi*. As seen from table 1, which lists the distinguishing characters of these different yeasts, the findings of van Uden and Assis-Lopes raise several interesting questions. In the first place, is melibiase synthesis in *S. italicus* var. *melibiosi* governed by a single gene or by polymeric genes, and, secondly, is this gene(s) identical with the Me-gene of *S. carlsbergensis*? Of added interest would be a comparison of the genetics of maltose and galactose fermentation in these two yeasts.

2. MATERIALS AND METHODS

For the purpose of investigating these problems a request for cultures was sent to Dr van Uden in Lisbon, who kindly supplied us with the following three strains of his new variety :

2613. From human fæces. Our Number 510
2624. From horse cæcum. Our Number 511
2625. From human fæces. Our Number 512.

In the work reported herein, Cultures 510 and 511 were employed together with the homozygous recessive single spore culture 303-9, which was descended from hybridisations involving *S. chevalieri*, *S. italicus* and *S. carlsbergensis* (Winge and Roberts, 1957) and which contained the recessive genes for the synthesis of melibiose, β-h-fructosidase, and maltase, and the "slow" gene for the synthesis of galactozymase ($mermg_s$).

For sporulation, Fowell's sodium acetate agar (Fowell, 1952) was employed with excellent results. Isolations of the single spores, as well as hybridisation experiments, were performed with the aid of a Zeiss gliding micromanipulator according to the standard Carlsberg isolation technique (Winge, 1935). The cultures were maintained in 8 per cent. Pilsner wort, which was also used for isolation work. The various sugars employed in the fermentation tests were added to yeast water at a concentration of 2 per cent. with the exception of raffinose, which was employed at 4 per cent. concentration. Qualitative fermentation tests on young dextrose-grown cultures were carried out singly in either Einhorn or Winge fermentometers, and when the sugar to be tested was melibiose, the cells were washed in sterile tap water and centrifuged before the analyses. In doubtful cases, the test was repeated and often rechecked in the van Iterson-Kluyver fermentometer, which was also employed for all quantitative determinations of fermentative ability.

In the test for melibiose fermentation, 1 c.c. of 2 per cent. melibiose yeast water was added to the culture immediately after washing, since it was noted that the time interval between washing and first contact with melibiose in the fermentometer could influence the readings, especially if this period was not of uniform duration for all cultures. When fermentation analyses are to be made on a large number of tetrads, it is most convenient to wash and centrifuge as many cultures as possible at the same time ; this necessarily results in a considerable lapse of time

between the completion of the first and the last analysis, during which the cells are suspended in a small amount of tap water. Since any considerable prolongation of time between washing and contact with melibiose may retard the onset of fermentation as much as several days, the addition of melibiose to the washed cells was found to be necessary. In this procedure, the amount of fermentation occurring before the suspended culture was placed in the fermentometer was found to be negligible. With respect to the fermentation analyses, it was also found to be advantageous in some cases to hasten the onset of fermentation by the addition of small amounts of dextrose (Spiegelman and Dunn, 1947 ; Suomalainen, Axelson and Oura, 1956). To ten parts of yeast water containing 2 per cent. of the sugar to be tested was added one part of 2 per cent. dextrose yeast water. This relatively small amount of dextrose was not capable of yielding visible evidence of fermentation when inoculated with a yeast culture, but in combination with another sugar, it clearly hastened the fermentation of the latter. Whenever mixtures of sugars were employed, fermentation medium lacking dextrose was also employed as a control.

In the tetrad analyses, asci showing abnormal segregation were infrequently encountered, but since they represented only a very small proportion of the total number analysed, they have been omitted from the tables and disregarded in determination of genotype.

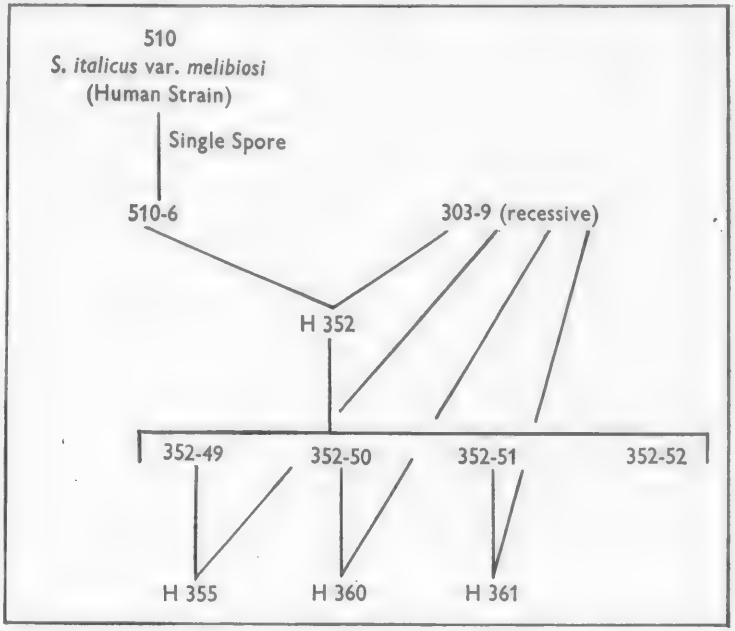
3. EXPERIMENTAL RESULTS

(1) *Analysis of* 510 (*human strain*)

The giant colony of the original strain of 510 presented a smooth surface apparently devoid of sectoring (plate I, fig. 2), while its single spore cultures segregated out in the form of dissimilar giant colonies, indicating that 510 was heterozygous. With respect to cellular morphology, a 2 : 2 segregation was found in 6 tetrads, half of the single spore cultures being composed of long cells and half of round-oval cells (plate I, figs. 6, 7). This is undoubtedly the same phenomenon already reported by Ditlevsen (1944) in a strain of *S. italicus* isolated from wine must. Single spore cultures from another 3 asci were then tested for their ability to ferment melibiose : all 12 cultures were fermenters. This absence of segregation indicated that (1) 510 was homozygous with respect to the melibiase gene (MeMe) or (2) 510 was either homozygous or heterozygous with respect to a number of polymeric melibiase genes.

It was decided to commence the hybridisation work with a self-diploidised single spore culture of 510 capable of fermenting melibiose, rather than with 510 itself. This would of course make it impossible to answer the question " How many melibiase genes does 510 have ? ", but in view of the possible complexity of the problem as indicated by (2) above, it was thought wiser first to determine how many genes are present in a melibiose-fermenting segregant. For this purpose the single spore culture 510-6 was chosen. It was long-celled and capable of fermenting melibiose, maltose and galactose. In order to be sure that this culture was normal with respect to homozygosity, it was brought to sporulation and 8 spores from two asci were isolated ; the resulting single spore cultures were found to be identical with respect to cellular morphology, appearance of the giant colony, and melibiose fermentative ability.

(a) *Hybrid 352.* The first hybridisation attempts involved 24 crossings between 510-6 and the recessive 303-9, resulting in the production of only one hybrid (H 352 in text-fig. 2), which, as was to be expected, was itself capable of fermenting melibiose, maltose and galactose. Sucrose was not fermented. In appearance, it resembled more 510-6 than 303-9 (plate I, fig. 1).



TEXT-FIG. 2.—The crossing experiments carried out for the purpose of determining the number of Me-genes in the single spore, 510-6.

Hybrid 352 possessed considerable fertility, the percentage of spore germination being 82·5. Thirty asci were dissected, and of these, 21 yielded complete tetrads. Sixteen were analysed for the ability to ferment maltose, and a 2 : 2 ratio was obtained in each ascus. This showed that only a single gene for maltase synthesis was present in 510-6. Seven of the 21 asci were analysed for galactose fermentation and as all yielded a 2 : 2 ratio, it appeared reasonably certain that 510-6 contained the G-gene. With respect to melibiose fermentation, however, the results were quite different. Ten asci segregated out as 4 : 0, 10 as 3 : 1, and one as 2 : 2, making a total of only 14·3 per cent. non-fermenters. Expressed as percentages, there were 47·6 per cent.-4 : 0, 47·6 per cent.-3 : 1, and 4·8 per cent.-2 : 2, as compared with the theoretical 3-gene segregation (50 per cent. crossing over) of 52·8 per cent.-4 : 0, 44·4 per cent.-3 : 1, and 2·8 per cent.-2 : 2, which

comprises 12·5 per cent. non-fermenters. Although this agreement was sufficiently close to warrant the assumption that 510-6 contained 3 polymeric Me-genes, it was evident that final proof of the assumption could be obtained only from backcrosses.

In the backcrossing experiments it was decided to attempt hybridisations between the recessive 303-9 and all of the single spore cultures of one of the 21 tetrads already analysed, since it was expected that this method would result in the most complete genetic analysis possible. If, for example, a 3 : 1 ascus of H 352 is chosen for backcrossing, and it is assumed that 3 Me-genes are present heterozygotically in H 352, then the 4 ascospores (I-IV) of this tetrad could be expected to contain 2, 2, 2, and 0 Me-genes or 3, 2, 1, and 0 Me-genes, as, *e.g.* in the following arrangement : *

I	II	III	IV
$Me_1Me_2Me_3$	$Me_1me_2Me_3$	$me_1Me_2me_3$	$me_1me_2me_3$

A 3 : 1 ascus from H 352 was chosen for this study ; its 4 single spore cultures were designated 352-49, -50, -51 and -52, of which 52 alone was unable to ferment melibiose.

(b) *Backcross Hybrid 355.* Eight crossing attempts were made between the recessive 303-9 and 352-49, which was able to ferment only melibiose and maltose, and one of these resulted in Hybrid 355 (text-fig. 2). Eighteen asci were dissected, and of these, 9 gave complete tetrads ; the spore germination percentage of H 355 was 83. Melibiose fermentation analyses of these 9 asci resulted in only 2 : 2 ratios, indicating that 352-49 must have had only one melibiase gene, as Spore III above. Maltose fermentation tests also yielded a 2 : 2 segregation, as expected.

(c) *Backcross Hybrid 360.* This hybridisation between the recessive 303-9 and 352-50, which fermented melibiose, maltose and galactose, proved to be difficult ; only one zygote was obtained out of a total of 52 spore pairings. This was designated Hybrid 360 (text-fig. 2), and its spore germination percentage was found to be 78. Only 10 complete asci were recovered from 24 dissected ones. Eight of them gave a 3 : 1 segregation with respect to melibiose fermentation, while the 2 remaining ones gave 4 : 0. This is in substantial agreement with a 2-gene segregation (50 per cent. crossing over), which theoretically would yield 6·7-3 : 1, 1·7-4 : 0 and 1·7-2 : 2 (66·7 per cent : 16·7 per cent. : 16·7 per cent.). It is therefore concluded that the melibiose fermenting 352-50 contained 2 Me-genes, as Spore II above. Both the maltose and the galactose fermentation analyses yielded 2 : 2 segregation ratios in all 10 asci ; these genetic markers demonstrate not only that one gene was present in 352-50 for maltase and one for galactozymase, but also that H 360 was a true hybrid.

* Here as well as in what follows the numerical subscripts of the different genes are arbitrarily chosen.

One of the 4 : 0 asci of H 360 (360-13 to 16) was selected for further study of the comparative fermentative ability of its 4 single spore cultures. Since it was known that two different Me-genes were distributed among these 4 spores, then 2 of them must contain the one gene, and 2, the other. Furthermore, this tetrad segregated out as 2 : 2 with respect to galactose fermentation, as follows :

360—13 Me_1g_s
—14 Me_1G
—15 Me_2G
—16 Me_2g_s

Quantitative raffinose and melibiose analyses were performed in the van Iterson-Kluyver fermentometer. The results showed that 360-14 and 360-15 fermented melibiose completely (due to the presence of the G-gene), while 360-13 and 360-16 fermented melibiose partially (due to the absence of the G-gene). Raffinose was fermented only by 360-14 and 360-15, since 360-13 and 360-16, although capable of hydrolysing the raffinose molecule, were unable to ferment it. After fermentation had taken place in a van Iterson-Kluyver fermentometer inoculated with 360-14 and containing raffinose yeast water, the sugar solution was tested chromatographically. By employing *n*-butanol-acetic acid as a solvent and *p*-anisidine-HCl as a spraying agent (Block, Le Strange and Zweig, 1952), it was found that the solution contained only sucrose. These chromatographic tests, in conjunction with the quantitative fermentation analyses, provide complete confirmation of the biochemical studies of Losada (1957) and the genetic studies of Winge and Roberts (1957) on the fermentation of raffinose by complementary gene action.

(d) *Backcross Hybrid 361.* Now if our assumption is correct that 510-6 contained 3 Me-genes, and knowing that 352-49 contained 1 Me-gene, that 352-50 contained 2 Me-genes, and that 352-52 is unable to ferment melibiose, then 352-51 must be similar to Spore I (p. 504) and contain all three Me-genes.

The hybridisation between the recessive 303-9 and 352-51, which fermented melibiose and galactose, proved to be the most difficult of all. Only one crossing was successful out of 74 attempts ; the resulting hybrid was designated H 361 (text-fig. 2). It proved to be of low fertility, only 48 per cent. of its spores germinating. Twelve complete tetrads were recovered out of a total of 52 dissected. These yielded with respect to melibiose fermentation 6-4 : 0 and 6-3 : 1, and it is concluded that this is representative of the 3-gene segregation with 50 per cent. crossing over (theoretically, 6·3-4 : 0, 5·3-3 : 1 and 0·3-2 : 2) ; the agreement is actually very close, in view of the small number of asci analysed. With respect to galactose fermentation, all 12 asci yielded the expected 2 : 2 segregation ratio.

(e) *Conclusions.* The results obtained above are summarised in table 2, where it will be seen that the genetic formulas of the 4 single

spore cultures from a 3 Me : 1 me ascus of H 352 can be derived, and, furthermore, that their derivation is in accord with theoretical expectation based on Mendelian law. Since three different polymeric genes for the synthesis of melibiase have been found to be present in one single spore from 510, it can be concluded that van Uden's human strain of *Saccharomyces italicus* v. *melibiosi* contains *at least* 3 polymeric Me-genes, which have been designated Me_1, Me_2 and Me_3. No evidence for the presence in this strain of more than one gene either for maltase or galactozymase synthesis was obtained.

TABLE 2

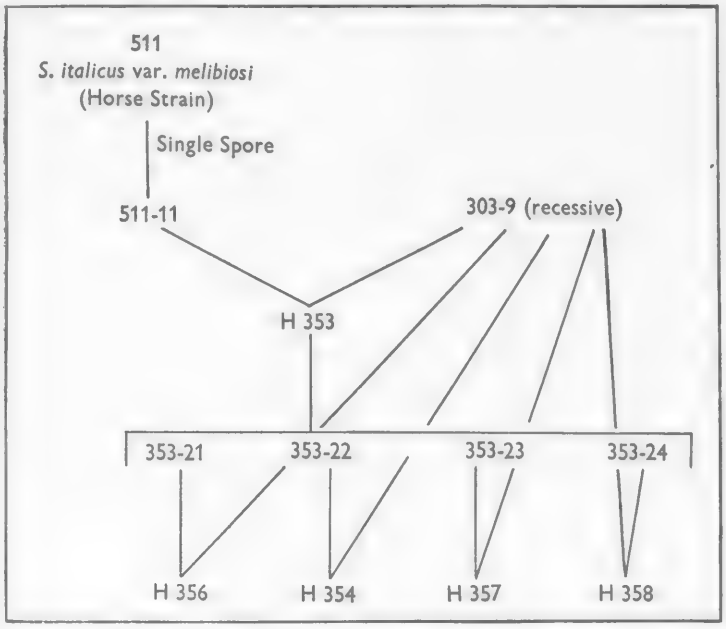
Table showing the method employed in determining the genotypes of the four single-spore cultures of the tetrad 352-49, -50, -51 and -52

Hybrid	Produced by crossing		Fermentation ratios of hybrid progeny			Derived formula of the unknown genotype
	Known genotype ×	Unknown genotype	Melibiose	Maltose	Galactose	
355	303-9 me m g_s	352-49 (ferments melibiose and maltose)	9-2 : 2	9-2 : 2	...	$Me_1me_2me_3$ M g_s
360	303-9 me m g_s	352-50 (ferments melibiose, maltose, and galactose)	2-4 : 0 8-3 : 1	10-2 : 2	10-2 : 2	$me_1Me_2Me_3$ M G
361	303-9 me m g_s	352-51 (ferments melibiose and galactose)	6-4 : 0 6-3 : 1	...	12-2 : 2	$Me_1Me_2Me_3$ m G
...		352-52 (non-fermenter of melibiose, maltose, and galactose)	...	...	...	$me_1me_2me_3$ m g_s

(ii) *Analysis of* 511 (*horse strain*)

The original culture of 511 yielded a smooth giant colony without visible sectoring (plate I, fig. 4), while its single spore cultures gave rise to different types of giant colonies, indicating the heterozygous nature of the original culture. In contrast to the segregation in cellular morphology exhibited by the human strain, the horse strain and all of its single spore progeny were composed of round-oval cells. Actively growing cultures of 511 did, however, include a small number of cells which enlarged considerably and then burst, indicating that some

physiological abnormality must be present in the strain. It can be mentioned here that subsequent investigations with single spores of 511 (to be described below) revealed three different manifestations of the bursting phenomenon : (1) an isolated spore swells considerably and eventually bursts (plate I, fig. 5), (2) an isolated spore germinates by the formation of an abnormal protuberance whose few attached buds eventually burst, and (3) an isolated spore germinates to form a normal colony of hundreds of cells, a few of which burst.

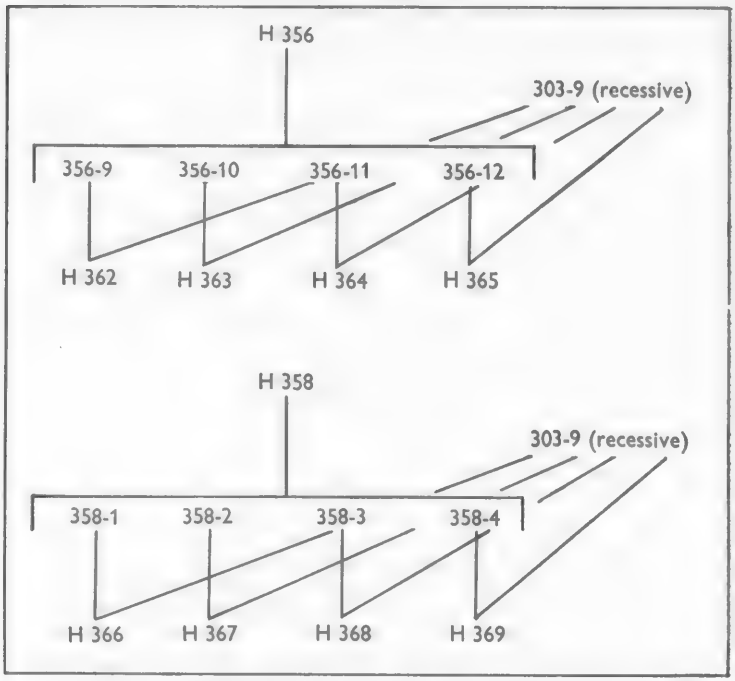


TEXT-FIG. 3.—A portion of the hybridisations necessary for gene number determination in the single spore, 511-11.

Preliminary experiments on segregation of fermentation characters were made on the cultures from 3 tetrads : galactose fermentation yielded 3-2 : 2 ratios, maltose fermentation 2-3 : 1 and 1-4 : 0, and melibiose, 12 fermenters. In the light of our experience with the human strain, these results indicated that the fermentation of melibiose, as well as maltose, by 511 was likewise governed by polymeric genes. It was also found that all 12 single spore cultures were capable of sporulation, probably due to the presence of the D-gene for diploidisation (Winge and Roberts, 1949).

(a) *Preliminary hybridisations.* Initial crossings were attempted between the original culture of 511 and the recessive 303-9 and led to the production of 2 hybrids, H 349, with a spore germination percentage of 68, and H 350, with a spore germination percentage of 73.

Tetrads derived from both hybrids yielded the following segregation ratios with respect to melibiose : H 349, 13-4 : 0 and H 350, 22-4 : 0 and 1-3 : 1. The fact that only one recessive culture was found among 144 tested indicated that the polymeric gene system occurring in 511 may include a greater number of genes than are present in 510. If, for example, 5 freely segregating Me-genes were present, then one would expect to find one recessive out of every 32 fermenters.



TEXT-FIG. 4.—Hybridisations necessary for gene number determination in H 356 and H 358.

Since a lower frequency of recessives actually was obtained in these preliminary analyses, it was assumed that the melibiase gene system in 511 must be of considerable complexity. For this reason, it was decided that, just as in the study of 510, it would be advisable first to determine the number of genes present in a single spore segregant of 511. For this purpose was chosen the diploid homozygous 511-11, a single spore culture which sporulated abundantly and which fermented melibiose, maltose and galactose. In order to be sure of the homozygous nature of this culture, 2 tetrads derived from it were analysed and were found to possess the parental characters with respect to fermentative ability and giant colony appearance.

(b) *Hybrid 353.* Only one crossing between 511-11 and 303-9 was successful out of 21 attempts. The resulting hybrid was designated H 353 (text-fig. 3), and it can be seen from fig. 3 of plate I that it resembled most the dominant parent. It was capable of fermenting melibiose, maltose and galactose and its spore germination percentage was 73.

Fourteen complete asci were recovered from a total of 40 dissected, and all 56 spores were able to ferment melibiose. That the hybrid itself was normal was shown by the 2 : 2 ratios obtained in 13 asci for both maltose and galactose segregation ; one ascus was abnormal, giving a 3 : 1 ratio for both maltose and galactose. The lack of segregation with respect to melibiose was unexpected, since it indicated that 511-11, just as the original 511, must contain a number of melibiase genes. It was therefore necessary to analyse a complete ascus of H 353 by attempting to backcross each of the 4 fermenting single spore cultures to the recessive in order to determine how many Me-genes each contained. For this purpose the ascus containing Spores 353-21, -22, -23 and -24 was selected (text-fig. 3) ; of these 4 single spore cultures, No. 22 appeared to ferment melibiose more slowly than the others.

(c) *Analysis of Ascus 353-21 to 24. Spore 353-21* (ferments melibiose and galactose). Eighteen crossing attempts between 353-21 and the recessive 303-9 resulted in one hybrid, H 356, which was capable of fermenting melibiose and galactose and which had a spore germination percentage of 65. Ten complete asci from H 356 were analysed for melibiose and galactose fermentation and yielded ratios of 5-4 : 0, 4-3 : 1 and 1-2 : 2 for melibiose and 10-2 : 2 for galactose. This segregation ratio for melibiose was in close agreement with the theoretical 3-gene segregation (5·3-4 : 0, 4·4-3 : 1 and 0·3-2 : 2), but in order to obtain additional confirmation of the existence of 3 melibiase genes in Spore 21, the following experiment was designed : From H 356 a 4 : 0 ascus was chosen for further hybridisation studies which would consist in backcrossing each of its 4 single spore cultures to the recessive for the purpose of determining whether H 356 actually had segregated out 3 genes in this ascus. If this proved to be the case, then the existence of 3 genes in Spore 21 would be confirmed.

Accordingly, the ascus 356-9, -10, -11 and -12, whose 4 single spore cultures all fermented melibiose, was selected (text-fig. 4). The details of the hybridisations are presented in table 3, where it will be seen that all 4 single spore cultures were not only able to hybridise successfully with the same recessive type but also that the hybrids possessed a high degree of fertility. These facts indicate that in this material the mating type system is either masked by the possible existence of the D-gene or is completely absent (Winge and Roberts, 1958).

With respect to melibiose fermentation, H 362 yielded a 2-gene segregation (8-3 : 1, 1-4 : 0 and 3-2 : 2 + 1 abnormal 0 : 4), H 363 yielded a 1-gene segregation (9-2 : 2 + 1 abnormal 0 : 4) and H 364,

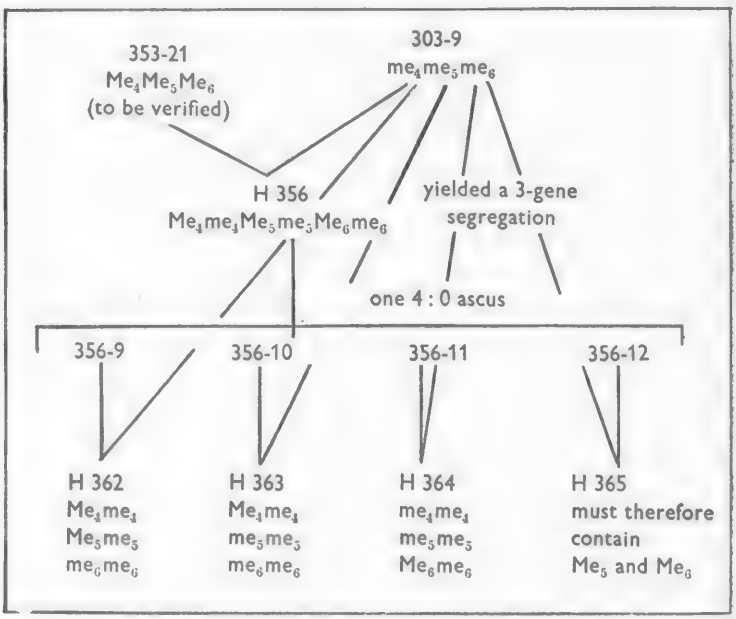
9-2 : 2. Segregations of 2 : 2 for galactose fermentation were obtained from H 362 and H 363. Now the fact that of the 4 spores to be tested,

TABLE 3

Hybridisations undertaken between a recessive and each of the four spores from one ascus for the purpose of determining the number of Me-genes in Spore 21

Parents	Crosses attempted	Crosses successful	Resulting hybrid	Hybrid asci dissected	Tetrads obtained	Spore germination per cent.
356-9 ×303-9	18	1	H 362	17	12	85
356-10×303-9	6	1	H 363	12	10	95
356-11×303-9	8	1	H 364	14	9	86
356-12×303-9	8	1	H 365	17	11	76

Nos. 9, 10 and 11 appeared to contain 2, 1 and 1 Me-genes respectively indicates that the remaining spore (12) must contain 2 Me-genes if



TEXT-FIG. 5.—Derivation of the number of Me-genes present in H 365.

the assumption that 353-21 contained 3 genes was correct. This is illustrated diagrammatically in text-fig. 5. Upon analysing the

progeny of H 365, confirmation of the existence of 3 Me-genes in 353-21 was obtained by the fact that H 365 itself gave a 2-gene segregation (8 asci yielded a 3 : 1 ratio and 3, a 4 : 0 ratio). These results also demonstrate that a genetic analysis of only 10 tetrads from H 356 was actually sufficient to determine accurately the number of Me-genes present in Spore 21.

Spore 353-22 (ferments melibiose). From text-fig. 3 it can be seen that H 354 was produced by hybridising 353-22 with the recessive. This hybrid, which had a spore germination percentage of 66, fermented melibiose but neither galactose nor maltose. Eight tetrads were analysed with respect to melibiose fermentation, and all yielded a 2 : 2 segregation, showing that only one Me-gene was present in Spore 22.

Spore 353-23 (ferments melibiose, maltose and galactose). Twelve crosses were attempted between 353-23 and 303-9, resulting in 2 hybrids. The first which was analysed showed no segregation with respect to maltose and galactose, indicating that the hybrid was not a true one, most probably due to some abnormality at the time of spore copulation. Furthermore, its spore germination percentage was only 47, and many spores and cells were observed to swell and burst.

The other hybrid, which was designated H 357 (text-fig. 3) had a spore germination percentage of 67·5. Twenty-two tetrads were analysed, of which 3 were abnormal with respect to segregation ratios for one or more of the 3 sugars and were discarded. Fifteen asci, however, gave 1-gene segregations for both maltose and galactose. Nineteen asci yielded the following ratios with respect to melibiose fermentation : 11-3 : 1 and 8-4 : 0. In contrast to the segregation from H 356, this segregation is neither characteristic of a 2-gene nor a 3-gene segregation. It resembles most, however, the latter (theoretically, 8·4-3 : 1 and 10-4 : 0), and Spore 23 was therefore assumed to have 3 Me-genes.

Spore 353-24 (ferments melibiose and maltose). If we are correct in assuming that the first 3 spores (21, 22, 23) of the tetrad 353-21 to 24 contain 3, 1 and 3 Me-genes respectively, then we can calculate in advance how many genes should theoretically be expected to occur in Spore 24, and, consequently, the number of genes in 511-11 (text-fig. 3). It was found that an even number of genes could not be present in Spore 24 (since the total number of Me-genes in the tetrad must be divisible by 2), but that 1, 3, 5 or 7 genes were possible. One possible gene arrangement is shown in table 4, which at the same time indicates the relationship between gene number in Spore 24 and in Spore 511-11. Expressed in another way, if the hybrid arising from the cross Spore 24 × the recessive yields a 1-gene segregation for melibiose, then 511-11 has 4 genes ; if a 3-gene segregation, then 511-11 has 5 genes ; 5-gene segregation, 6 genes ; and 7-gene segregation, 7 genes.

A hybrid was produced between the single spore culture arising from Spore 24, 353-24, and the recessive and was designated as H 358

(text-fig. 3). Its spore germination percentage was 69, and 9 complete tetrads were obtained. These were analysed with respect to melibiose fermentation and all 36 cultures were capable of fermentation. Although this result was a strong indication of the presence of many Me-genes in Spore 24, nothing more definite could be deduced from it. It was therefore again necessary to backcross a complete tetrad of H 358 and analyse all 4 hybrids in the usual way in order to determine gene

TABLE 4

Relationship between gene number in Spore 24 and in Spore 511-11

Spore	No. of Me-genes	511-11 with 4 Me-genes	511-11 with 5 Me-genes	511-11 with 6 Me-genes	511-11 with 7 Me-genes
21	3	$Me_1Me_3Me_4me_7$	$Me_1Me_3Me_4me_5me_8$	$Me_1Me_3Me_4me_5me_6me_8$	$Me_1Me_3Me_4me_5me_6me_{11}$
22	1	$Me_1me_3me_4me_7$	$Me_1me_3me_4me_5me_8$	$Me_1me_3me_4me_5me_6Me_8$	$me_1me_3me_4me_5me_6Me_{11}$
23	3	$me_1Me_3Me_4Me_7$	$me_1me_3Me_4Me_5Me_8$	$me_1me_3Me_4Me_5Me_6Me_8$	$me_1me_3Me_4me_5Me_6Me_{11}$
24	To be determined	$me_1me_3me_4Me_7$	$me_1Me_3me_4Me_5Me_8$	$Me_1Me_3Me_4Me_5Me_6me_8$	$Me_1Me_3Me_4Me_5Me_6Me_{11}$
		∴ 1 gene in Spore 24	∴ 3 genes in Spore 24	∴ 5 genes in Spore 24	∴ 7 genes in Spore 24

number in Spore 24. The details of the hybridisations are presented in table 5 ; here again the success of the crossing attempts confirms the absence of a functional mating system in this material.

Spore 358-1 (ferments melibiose). The spore germination of H 366 (text-fig. 4) was complicated by the bursting phenomenon. In 43 of the 72 asci 2 of the spores swelled and then burst, whereas the 2 remaining spores germinated normally. In only 3 asci did all 4 spores

TABLE 5

Hybridisations undertaken between a recessive and each of the four spores from one ascus for the purpose of determining the number of Me-genes in Spore 24

Parents	Crosses attempted	Crosses successful	Resulting hybrid	Hybrid asci dissected	Tetrads obtained	Spore germination per cent.
358-1 × 303-9	30	1	H 366	72	3	48
358-2 × 303-9	26	1	H 367	24	10	72·4
358-3 × 303-9	12	1	H 368	31	13	67
358-4 × 303-9	8	1	H 369	43	21	68·8

germinate normally. In the remaining 26 asci, incomplete germination or cell bursting, or both, occurred.

The 3 complete tetrads yielded a 2 : 2 segregation with respect to melibiose. Neither maltose nor galactose gene markers occurred in this hybrid. Sixteen spore cultures derived from 8 incomplete asci were also analysed, but no indication was found of linkage between melibiose fermentation and the bursting phenomenon.

On the basis of these few data, we assume that Spore I contains only one gene for melibiase synthesis.

A few experiments were carried out * on the effect of increased osmotic pressure on the bursting phenomenon in the spores. Dissections of asci of H 366 were performed in 10 different concentrations of dextrose yeast water ranging from 3-12 per cent.; 3 asci were dissected in each concentration. Concentrations up to and including 10 per cent. were unable to prevent bursting, while the only effect of 11 and 12 per cent. concentrations was that the swollen spores remained intact for a longer period of time (48 hours or more) before their ultimate collapse. No indication of budding was observed in the swollen spores.

Spore 358-2 (ferments melibiose and maltose). Hybrid 367 (text-fig. 4) was produced by crossing 358-2 and 303-9 and had a spore germination percentage of 72·4. The 10 complete asci which were analysed yielded a 2-gene segregation (6-3 : 1, 3-4 : 0 and 1-2 : 2) for melibiose fermentation and a clear 2 : 2 segregation for maltose fermentation. Spore 358-2 is thus assumed to possess 2 different Me-genes.

Spore 358-3 (ferments melibiose and maltose). Hybrid 368 (text-fig. 4) was produced by crossing 358-3 and 303-9 and had a spore germination percentage of 67. The 13 complete asci which were analysed yielded 6-3 : 1, 6-4 : 0 and 1-2 : 2 for melibiose fermentation and only 2 : 2 segregations for maltose fermentation. Although the melibiose segregation ratios obtained were not typical, they showed more resemblance to the theoretical 3-gene segregation than to any other, and for this reason it was assumed that Spore 358-3 contained 3 different Me-genes.

Spore 358-4 (ferments melibiose). If the above determinations of gene number are correct, then the first 3 spores of the ascus (358-1, -2, -3) contain 1, 2 and 3 Me-genes respectively. On the basis of these determinations, the last spore (358-4) could contain only 2, 4 or 6 genes, yielding 4-, 5- and 6-gene segregations respectively.

Hybrid 369 (text-fig. 4) was produced between 358-4 and 303-9 and had a spore germination percentage of 68·8. The 22 complete asci which were analysed for melibiose fermentation ability yielded 16-4 : 0 and 6-3 : 1 segregations. This result, while at once eliminating the possibility of either 2 or 6 genes, as mentioned above, demonstrates the presence of 4 genes in Spore 358-4. The theoretical 4-gene segregation (50 per cent. crossing over) would consist of 16·6-4 : 0, and 5·3-3 : 1, and the agreement is thus nearly perfect.

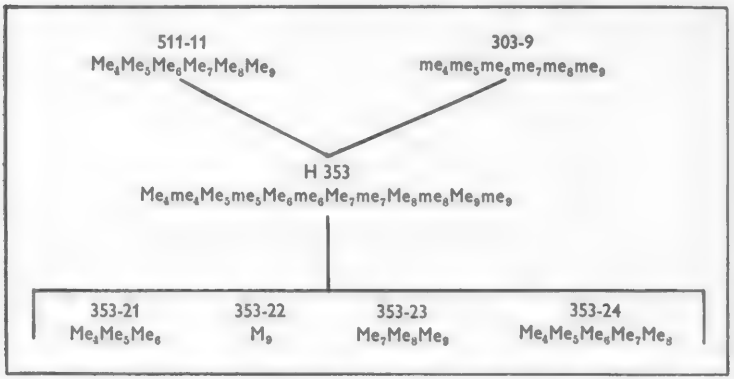
The derived genotypes of the 4 spores from the single 4 : 0 ascus of H 358 can now be written, for example, as :

358-1	$Me_4me_5me_6me_7me_8$
358-2	$Me_4Me_5me_6me_7me_8$
358-3	$me_4me_5Me_6Me_7Me_8$
358-4	$me_4Me_5Me_6Me_7Me_8$

* At the suggestion of Professor Øjvind Winge.

Knowing that 5 genes are thus present in H 358, the Spore 353-24 must necessarily have contained the same 5 genes, since it was crossed with the recessive to produce H 358 (text-fig. 3). And knowing that 353-24 contains 5 genes, then H 353 must necessarily have contained 6 genes, as shown in text-fig. 6. Finally, we are in a position to state that because H 353 has 6 genes, then these same genes must also have been present in the Spore 511-11.

(d) *Conclusions*. The genetic analyses reported above, the results of which are in full agreement with Mendelian law, demonstrate that van Uden's horse strain of *S. italicus* var. *melibiosi* contains *at least* 6 different polymeric genes for the synthesis of melibiase. These have



TEX -FIG. 6.—Derivation of the number of Me-genes in H 353 and the single Spore, 511-11.

been designated Me_4, Me_5, Me_6, Me_7, Me_8 and Me_9, since it is not yet known whether any of them are identical with the Me_1, Me_2 and Me_3 genes present in the human strain. No evidence for the presence of more than one gene for the synthesis of galactozymase was obtained, but the strain appears to have more than one gene for the synthesis of maltase.

4. DISCUSSION

This study was prompted by two unanswered questions : (1) How many melibiase genes are present in *S. italicus* var. *melibiosi*? and (2) Is the one melibiase gene present in *S. carlsbergensis* identical with one of the genes in *S. italicus* var. *melibiosi*? These questions have not been answered. The elucidation of the genetics of melibiose fermentation in this yeast was found to be both a laborious and difficult task due to the existence of polymeric genes in the material. On the basis of several hundred tetrads we have, however, been able to demonstrate (1) that the human strain of *S. italicus* var. *melibiosi* contains at least 3 freely segregating Me-genes, (2) that the horse strain of *S. italicus* var. *melibiosi* contains at least 6 apparently unlinked Me-genes, (3) that the mechanism of raffinose hydrolysis in the absence of β-h-fructosidase

is precisely the same in both *S. italicus* var. *melibiosi* and *S. carlsbergensis*, and (4) that there is no evidence in either strain for the existence of polymeric genes involved in the fermentation of galactose, but that it is likely that the horse strain contains more than one gene for the synthesis of maltase.

It is interesting to compare the genic differences among the yeasts under consideration, which are listed, together with their habitats, in table 6. It should not be overlooked, however, that it is theoretically possible for the human strain to contain the same number of Me-genes as the horse strain ; in the present state of our knowledge we can only be sure that the former contains at least 3. Since these polymeric Me-genes segregate out in crosses, we know that they must be situated

TABLE 6

The yeasts under discussion compared with respect to habitat and number of melibiase genes present

Yeast	Primary habitat	Number of melibiase genes present
S. carlsbergensis . . .	Grape must and juice, bottom fermentation in breweries	1
S. italicus	Grape must	0
S. italicus var. *melibiosi* . .	Digestive tract of man	3 or more
S. italicus var. *melibiosi* . .	Digestive tract of horse	6 or more

at different loci, but we are not yet in a position to know whether they are biochemically identical. If they are identical entities, the possibility exists that they may have arisen through chromosome duplication or inversion. But whatever their origin, the fact remains that only a single polymeric gene is sufficient for the synthesis of the enzyme which governs the hydrolysis of the sugar in question. *S. carlsbergensis*, with its one Me-gene, ferments melibiose just as does the horse strain of *S. italicus* var. *melibiosi*, with its six or more Me-genes, while a genetic segregant of the horse strain containing only one Me-gene appears no less effective in fermenting melibiose than the original multigenic strain.

Since the standard system of classification of the yeast fungi (Lodder and Kreger-van Rij, 1952) rightly places so much emphasis upon fermentative ability, it would seem that determination of the fermentative genotype would be a highly desirable undertaking for the future. From an ideal point of view, knowledge of the number and kinds of fermentative genes occurring in any particular yeast could be regarded as a valuable supplement to its diagnostic characters, since such genetic knowledge, if sufficiently embracing in scope, might be instrumental in the attainment of a more natural system of classification. However, the difficulties that are involved in such a project are well illustrated in the present study which, although laborious and detailed,

yielded only preliminary factual knowledge of the genetics of melibiose fermentation in the two strains employed : the actual number and identity of the melibiase genes present have not been determined. When only one gene for a particular fermentative character occurs, the problem of isolating and identifying it is, of course, immeasurably simpler, but the increasing number of recorded instances in yeasts of polymeric genes leads one to the conclusion that their taxonomic value alone is not sufficient to justify the nearly insurmountable work required for their complete elucidation.

Finally, a word should be added on the subject of asci which yielded unexpected segregation ratios. For example, in the horse strain, 6 abnormal asci were obtained out of a total of 160 analysed. This figure of less than 4 per cent. we consider to be an expression of the exceptions or abnormalities which are so frequently encountered in biological material and have accordingly made no attempt to investigate them further.

5. SUMMARY

A genetic investigation of *Saccharomyces italicus* var. *melibiosi* has revealed the presence of at least 3 polymeric melibiase genes in the strain isolated from the digestive tract of man and at least 6 polymeric melibiase genes in the strain isolated from the digestive tract of horse. Whether any of the genes from the one strain are identical with those of the other has not been determined.

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Plate

FIG. 1.—Giant colonies of H 352 and its parents, 510-6 (left) and 303-9 (right).

FIG. 2.—Giant colony of the original human strain, 510.

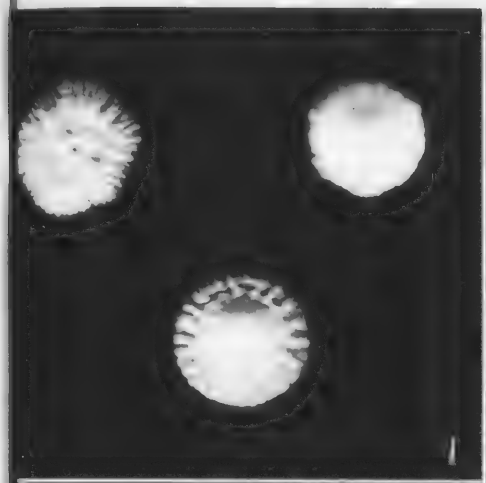
FIG. 3.—Giant colonies of H 353 and its parents, 511-11 (left) and 303-9 (right).

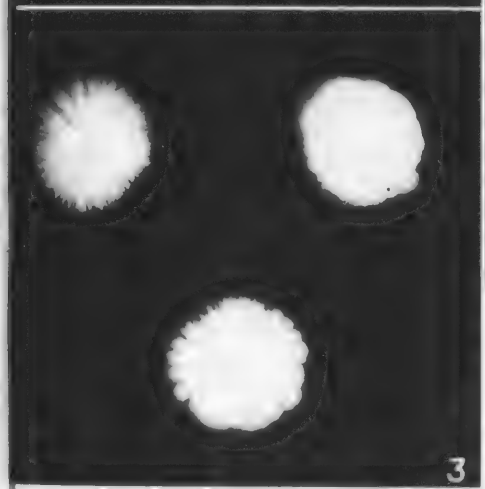
FIG. 4.—Giant colony of the original horse strain, 511.

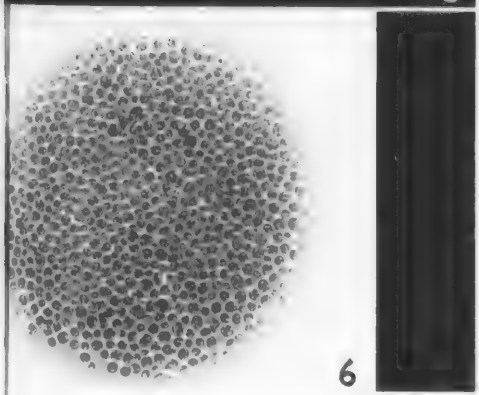
FIG. 5.—An isolated spore of H 366 which has burst. Photographed in a wort droplet in the dissecting chamber. (*ca.* 2500×.)

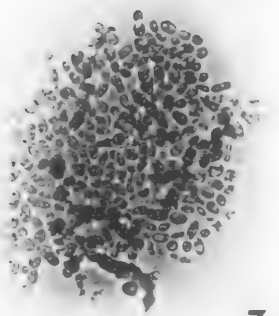
FIG. 6.—A colony composed of round-oval cells derived from a single spore of 510. Photographed in a wort droplet in the dissection chamber. (*ca.* 1500×.)

FIG. 7.—A colony composed of long cells derived from a single spore of 510. Photographed in a wort droplet in the dissecting chamber. (*ca.* 1500×).









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NOTES AND COMMENTS

AN INEQUALITY IN POPULATION GENETICS

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1. INTRODUCTION

In the theory of population genetics, it is well known that genic equilibrium is possible among a system of alleles at a single locus in a large random mating population, when there is differential viability between the various possible genotypes. In examining the stability of such an equilibrium with respect to chance fluctuations of the gene frequencies (Mandel, 1959) it has been found useful to consider the change in mean viability of the population from one generation to the next. In the case where generations overlap or when selection is infinitesimally slow it is fairly easy to show, by introducing a continuous time parameter, that the mean viability must increase with time (Kimura, 1956 ; Crow and Kimura, 1956). When the generations are discrete and non-overlapping the problem is more difficult mathematically, and gives rise to an inequality ((21) later) which does not appear to have been proved before. The object of this paper is to establish this inequality and to give a brief account of the way in which it arises out of genetical considerations.

2. STATEMENT OF THE PROBLEM

Consider n alleles A_1, A_2, ..., A_n at a single genetic locus. Let $a_{ij} \geqslant 0$ denote the relative viability of the genotype A_iA_j $(i, j = 1, 2, \ldots, n)$; the a_{ij} thus form a symmetric $n \times n$ matrix (a_{ij}) with non-negative elements. Let p_i be the frequency of gene A_i $(i = 1, 2, \ldots, n)$ in any one generation so that

$$\sum_{i=1}^{n} p_i = 1, \quad 0 < p_i < 1. \tag{1}$$

Let

$$V = \sum_{i=1}^{n} \sum_{j=1}^{n} a_{ij} p_i p_j. \tag{2}$$

V is called the mean viability of the population.

If random mating takes place among the individuals of this generation, the frequencies p_i' of the alleles A_i in the next generation are given by the relations

$$V p_i' = p_i \sum_{j=1}^{n} a_{ij} p_j \quad (\text{all } i = 1, 2, \ldots, n). \tag{3}$$

The mean viability V′ of the population in this generation is

$$V' = \sum_{i=1}^{n} \sum_{j=1}^{n} a_{ij} p_i' p_j'. \tag{4}$$

Under certain conditions (see, *e.g.* Mandel, 1959, it is possible for the alleles A_i to be maintained in polymorphic equilibrium, *i.e.* for the gene frequencies p_i to remain constant from generation to generation, with $p_i > 0$ for all i. It has been shown (*ibid.* and Mandel and Hughes, 1958) that when the population is in the neighbourhood of such an equilibrium state the inequality

$$V' - V \geqslant 0 \tag{5}$$

is true. This result enables the stability of the equilibrium to be investigated. It has been suspected, and will be proved here, that (5) holds for all states of the population, independently of the restriction imposed above.

In the two allele case $n = 2$, it is possible to obtain, by elementary but heavy algebra, an explicit expression for the difference V'-V, viz.

$$V' - V = \frac{1}{V^2}\{p_1(a_{11}p_1 + a_{12}p_2 - V)^2 + p_2(a_{12}p_1 + a_{22}p_2 - V)^2\} \{V + a_{11}p_1 + a_{22}p_2\} \tag{6}$$

and this clearly establishes the validity of (5). The relation (6), however, does not appear to admit any generalisation to $n \geqslant 3$ and hence the following approach is adopted.

3. PROOF OF THE INEQUALITY

Letting

$$\Phi = (a_{ij}) \quad (i, j = 1, 2, \ldots, n) \tag{7}$$

$$P = \text{diag}\,(p_i) \quad (i = 1, 2, \ldots, n) \tag{8}$$

and

$$R^T = (p_1, p_2, \ldots, p_n) \tag{9}$$

we have

$$V = R^T \Phi R. \tag{10}$$

Also

$$R'^T = (p_1', p_2', \ldots, p_n') = \frac{P\Phi R}{V} \tag{11}$$

and

$$V' = R'^T \Phi R' = \frac{1}{V^2} R^T \Phi P \Phi P \Phi R. \tag{12}$$

It is convenient now to represent the state of the population not by the numbers p_i but by $\sqrt{p_i}$; the vector

$$Q^T = (\sqrt{p_1}, \sqrt{p_2}, \ldots, \sqrt{p_n}) \tag{13}$$

is then a vector with unit norm.

We also define the matrix

$$S = \text{diag}\,(\sqrt{p_i}) \quad (i = 1, 2, \ldots, n) \tag{14}$$

so that

$$S^2 = P \tag{15}$$

and the matrix

$$\Psi = S\Phi S. \tag{16}$$

Since Φ is symmetric, so also is Ψ. Noting that

$$R = SQ \tag{17}$$

we obtain from (10), (12) and (16)

$$V = Q^T S \Phi S Q = Q^T \Psi Q \tag{18}$$

$$V' = \frac{1}{V^2} Q^T S \Phi S^2 \Phi S^2 \Phi S Q = \frac{1}{V^2} Q^T \Psi^3 Q. \tag{19}$$

Thus in order to prove (5) it must be shown that, for any real symmetric matrix Ψ with non-negative elements, and any vector Q with positive elements and unit norm

$$Q^T \Psi^3 Q \geqslant (Q^T \Psi Q)^3. \tag{20}$$

We shall actually prove the slightly more general inequality

$$Q^T \Psi^r Q \geqslant (Q^T \Psi Q)^r \tag{21}$$

under the same restrictions, for all integral $r > 0$.

Let the matrix Ψ have eigenvalues $\alpha_i (i = 1, 2, \ldots, n)$ and let the components of Q referred to a set of mutually orthogonal eigenvectors of Ψ as a base be $X_i (i = 1, 2, \ldots, n)$. Since Ψ is symmetric, all the α_i are real.

We now invoke the following theorem due to Frobenius (1908, 1909, 1912).

3.1. *Theorem.* If Ψ is a matrix with non-negative elements, and $\Psi \neq 0$, then there is an eigenvalue, α_1 say, of Ψ with the following properties :

(i) α_1 corresponds to an eigenvector of Ψ which has non-negative components when referred to the axes of Q;

(ii) $\alpha_1 > 0$; (22)

(iii) $\alpha_1 \geqslant |\alpha_1|$ (all $i = 1, 2, \ldots, n$). (23)

[For a particularly lucid discussion of these results see Debreu and Herstein (1953) ; also Wielandt (1950), Perron (1907).]

Suppose now that (21) holds when Q has $(n-1)$ components and Ψ is an $(n-1) \times (n-1)$ matrix.

3.2. *Lemma.* Then (21) also holds on the boundaries of the region for which $q_i > 0$, *i.e.* it holds when one of the q_i is zero.

Proof. Suppose that $q_n = 0$. Then

$$Q^T \Psi^r Q = (q_1, q_2, \ldots, q_{n-1}, 0 \left\{ \begin{pmatrix} \psi_{11} & \psi_{12} & \cdots & \psi_{1,n-1} & 0 \\ \psi_{21} & \psi_{22} & \cdots & \psi_{2,n-1} & 0 \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ \psi_{n-1,1} & \psi_{n-1,2} & \cdots & \psi_{n-1,n-1} & 0 \\ 0 & 0 & \cdots & 0 & 0 \end{pmatrix} \right.$$

$$\left. + \begin{pmatrix} 0 & 0 & \cdots & 0 & \psi_{1n} \\ 0 & 0 & \cdots & 0 & \psi_{2n} \\ \cdots & \cdots & \cdots & \cdots & \cdots \\ 0 & 0 & \cdots & 0 & \psi_{n-1,n} \\ \psi_{n1} & \psi_{n2} & \cdots & \psi_{n,n-1} & \psi_{nn} \end{pmatrix} \right\}^r \begin{pmatrix} q_1 \\ q_2 \\ \vdots \\ q_{n-1} \\ 0 \end{pmatrix}$$

$$\geqslant (q_1, q_2 \cdots q_{n-1}) \begin{pmatrix} \psi_{11} & \psi_{12} & \cdots & \psi_{1,n-1} \\ \psi_{21} & \psi_{22} & \cdots & \psi_{2,n-1} \\ \cdots & \cdots & \cdots & \cdots \\ \psi_{n-1,1} & \psi_{n-1,2} & \cdots & \psi_{n-1,n-1} \end{pmatrix}^r \begin{pmatrix} q_1 \\ q_2 \\ \vdots \\ q_{n-1} \end{pmatrix} \tag{24}$$

the extra terms being all non-negative, since the q_i and ψ_{ij} are all non-negative.

Furthermore

$$Q^T\Psi Q = (q_1, q_2, \ldots, q_{n-1}, 0) \begin{pmatrix} \psi_{11} & \psi_{12} & \cdots & \psi_{1n} \\ \psi_{21} & \psi_{22} & \cdots & \psi_{2n} \\ \cdots & \cdots & \cdots & \cdots \\ \psi_{n1} & \psi_{n2} & \cdots & \psi_{nn} \end{pmatrix} \begin{pmatrix} q_1 \\ q_2 \\ \vdots \\ q_{n-1} \\ 0 \end{pmatrix}$$

$$= (q_1, q_2, \ldots, q_{n-1}) \begin{pmatrix} \psi_{11} & \psi_{12} & \cdots & \psi_{1,\,n-1} \\ \psi_{21} & \psi_{22} & \cdots & \psi_{2,\,n-1} \\ \cdots & \cdots & \cdots & \cdots \\ \psi_{n-1,\,1} & \psi_{n-1,\,2} & \cdots & \psi_{n-1,\,n-1} \end{pmatrix} \begin{pmatrix} q_1 \\ q_2 \\ \vdots \\ q_{n-1} \end{pmatrix} \quad (25)$$

Thus, using (24), (25) and the inductive hypothesis, the inequality $Q^T\Psi^r Q \geqslant (Q^T\Psi Q)^r$ holds when $q_n = 0$, which proves the lemma.

Writing

$$F = Q^T\Psi^r Q - (Q^T\Psi Q)^r \quad (26)$$

we have

$$F \geqslant 0 \quad (27)$$

on the boundaries of the region $q_i > 0$.

Suppose now that $F < 0$ for some Q inside the region $q_i > 0$. Then there must be a minimum of F inside the region (for F is bounded) which has to be a stationary value for F, since the derivatives of F with respect to the q_i exist and are continuous. We therefore examine the stationary values of F.

Expressing Ψ and Q with the eigenvectors of Ψ as base, we have

$$F = \sum_{i=1}^{n} X_i^2 a_i^r - \Big(\sum_{i=1}^{n} X_i^2 a_i\Big)^r \quad (28)$$

with

$$\sum_{i=1}^{n} X_i^2 = |Q|^2 = 1. \quad (29)$$

Using Lagrange's method of undetermined multipliers, there must exist a λ such that

$$\frac{\partial F}{\partial X_i} - \partial \frac{\partial}{\partial X_i} \Big\{\sum_{i=1}^{n} X_i^2 - 1\Big\} = 2X_i a_i^r - r\Big\{\sum_{i=1}^{n} X_i^2 a_i\Big\}.\ 2X_i a_i - 2\lambda X_i = 0 \quad (30)$$

for all $i = 1, 2, \ldots, n$.

Hence, for all i, either

$$X_i = 0 \quad (31)$$

or

$$a_i^r - r a_i \Big\{\sum_{i=1}^{n} a_i X_i^2\Big\}^{r-1} = \lambda. \quad (32)$$

If $X_1 = 0$, the vector Q would be orthogonal to the vector whose components are (1, 0, 0, ..., 0) when referred to the eigenvectors of Ψ. But this is the eigenvector of Ψ corresponding to the eigenvalue a_1 with non-negative elements when referred to the original set of axes. We would then have $q_i \leqslant 0$ for some i, and hence Q falls outside the region in question.

Therefore

$$X_i \neq 0 \quad (33)$$

and (32) holds at least for $i = 1$.

Multiplying the latter half of (30) by $\frac{1}{2}X_i$ and summing over i,

$$\sum_{i=1}^{n} X_i^2 a_i^r - r\{\sum_{i=1}^{n} X_i^2 a_i\}^r = \lambda \sum_{i=1}^{n} X_i^2 = \lambda \tag{34}$$

i.e.

$$F = \lambda + (r-1)\{\sum_{i=1}^{n} X_i^2 a_i\}^r. \tag{35}$$

Hence, noting that

$$\sum_{i=1}^{n} a_i X_i^2 = Q^T \Psi Q = V > 0 \tag{36}$$

we have

$$\frac{F}{V^r} = \frac{\lambda}{V^r} + (r-1) = \frac{{a_1}^r - r a_1 V^{r-1}}{V^r} + (r-1) \tag{37}$$

using (32).

Thus

$$\frac{F}{V^r} = \left(\frac{a_i}{V}\right)^r - r\left(\frac{a_i}{V}\right) + (r-1) \tag{38}$$

Moreover, using (23),

$$V = \sum_{i=1}^{n} a_i X_i^2 \leqslant \sum_{i=1}^{n} a_1 X^2 = a_1. \tag{39}$$

Thus (38) may be written

$$\begin{aligned}\frac{F^r}{V} &= \left(\frac{a_1}{V}\right)^r - \left(\frac{a_1}{V}\right) - (r-1)\left\{\frac{a_1}{V} - 1\right\} \\ &= \left(\frac{a_1}{V} - 1\right)\left\{\frac{a_1}{V} + \left(\frac{a_1}{V}\right)^2 + \ldots + \left(\frac{a_1}{V}\right)^{r-1}\right\} - (r-1)\left\{\frac{a_1}{V} - 1\right\} \\ &= \left(\frac{a_1}{V} - 1\right)\left\{\frac{a_1}{V} + \left(\frac{a_1}{V}\right)^2 + \ldots + \left(\frac{a_1}{V}\right)^{r-1} - (r-1)\right\} \geqslant 0, \text{ by (39),}\end{aligned}$$

and therefore

$$F \geqslant 0 \tag{40}$$

holds throughout the region $q_i > 0$ and on its boundaries.

Thus (21) is true in n dimensions if it is true in $(n-1)$. But (21) holds (with equality) for $n = 1$, when Q has the single component 1 and Ψ the single element $\psi_{11} > 0$. Hence (21) is established for all n.

4. DISCUSSION

The inequality obtained above is interesting from two points of view. Genetically, in the form (5), it shows that the multiple allelic system described in 1., starting out from any initial state in which all the alleles are represented, will tend monotonically under natural selection towards a state of maximum mean viability. Mathematically, the inequality, represented in the form (28), (40), is well known (see Hardy, Littlewood and Polya (1934), p. 26) when the a_1 are non-negative. Here the inequality has been established for a different class of a_1, viz. the latent roots of real, symmetric non-negative matrices.

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THE GENETIC BASIS OF "DUALITY" IN *IMPERFECT* FUNGI

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The cytoplasmic basis of a heritable difference in phenotype is inferred from its failure to be transmitted from parent to offspring in the manner expected of a nuclear controlled difference. This generally means non-Mendelian inheritance, of which reciprocal differences are the simplest evidence. In some fungi reciprocal crosses have been used to distinguish between differences of nuclear and cytoplasmic origin (Mitchell and Mitchell, 1952 ; Day, 1959) but in most species the life cycle makes it necessary to draw this distinction without the aid of either reciprocal crosses or even of sexual reproduction. In these species we must rely on expectations derived from other properties of nuclear controlled differences to effect this separation.

In heterokaryons the component nuclei sort out as units in the production of asexual spores without any genic recombination between them. Hence each kind of nucleus can be marked and followed unambiguously in asexual spore production by a single mutant gene. If, therefore, in the asexual progeny of a heterokaryon a difference in phenotype fails to remain in association with the nuclear marker with which it entered the heterokaryon then the difference must be under cytoplasmic control (Jinks, 1954, 1956, 1958, 1959 ; Pittenger, 1956 ; Gowdridge, 1956 ; Arlett, 1957 ; Gibson and Griffin, 1958 ; Mahony and Wilkie, 1958 ; Roper, 1958 ; Sharpe, 1958). A single difference in phenotype between two homokaryon isolates cannot be classified directly by this test. Nor can the test be successfully used with two independently occurring differences in phenotype between the components of the heterokaryon unless one is known to be nuclear in origin. However, additional expectations for nuclear controlled differences can be derived from cytological investigations. Cytology can trace back the history of the nuclei of the asexual spores of a heterokaryon and tell us whether to expect only homokaryotic spores, or if heterokaryotic spores can be produced, their maximum frequency. It can further tell us whether all the spores in the same chain or head should have identical genotypes (Baker, 1944 ; Pontecorvo and Gemmell, 1944 ; Baker, 1945 ; Miller, 1946 ; Rees and Jinks, 1952; Pontecorvo, 1953 ; Buxton, 1954; Ishitani and Sakaguchi, 1955*a*, *b* ; Gibson and Griffin, 1958). Hence, failure of a difference in phenotype to behave according to these expectations would rule out a nuclear origin. On the other hand, agreement with these expectations is not in all cases unambiguous evidence of a nuclear origin.

In sexual species cytoplasmic differences are known which segregate at asexual spore formation in a manner analogous to the sorting out of whole nuclei. This leads to a segregation pattern in the asexual progeny which is similar to that of a heterokaryon producing a high proportion of heterokaryotic spores (Pittenger, 1956 ; Arlett, 1957 ; Catcheside, 1958 ; Gibson and Griffin, 1958 ; Jinks, 1958, 1959). There is no reason to believe that

similar cytoplasmic differences are not also present in *imperfect* fungi. Thus in those heterokaryons where we expect the production of a high proportion of heterokaryotic asexual spores, to observe a segregation in the asexual progeny compatible with this expectation is not proof of a nuclear origin.

If we apply these criteria to the many investigations into the nature of the wild variation in the imperfect fungi few can still be regarded as providing conclusive evidence as to its causation. Among those which do are the demonstrations of heterokaryosis in *imperfect Penicillium* (Jinks, 1952*a*, *b* ; Rees and Jinks, 1952), *Aspergillus* (Ishitani and Sakaguchi, 1955*a*, *b*) and *Fusarium* (Buxton, 1954) species. In these examples the complete accord between the detailed cytological evidence and the segregations observed in the asexual progeny of the heterokaryon leave no doubt as to the nuclear origin of the variation.

The " dual phenomenon " which is probably the most widely reported type of variation in the *imperfect* fungi has generally been attributed to the nuclei (Hansen and Smith, 1932 ; Hansen, 1938, 1942 ; Hansen and Snyder, 1944). The regularity of the segregation of the asexual progenies of " dual " fungi led Hansen to suggest that the " duality " sprang from the persistent association of different nuclei. This explanation has been generally accepted, but its acceptance has rested largely on a correlation claimed between the proportion of asexual spores of a " dual " colony which give rise to " dual " colonies ; the number of distinct phenotypes among these " dual " colonies ; and the number of nuclei in the asexual spores. While this correlation may have a general validity such evidence would be more compelling if, as suggested earlier, the origin of the nuclei in the spores was also known. Thus where all the nuclei of a single multinucleate or multicellular spore produced by a heterokaryon arise from the mitotic division of a single nucleus (Baker, 1945 ; Buxton, 1954 ; Ishitani and Sakaguchi, 1955*b* ; Gibson and Griffin, 1958) a " duality " based on heterokaryosis can no more be transmitted through single asexual spores than in those species of fungi which produce only uninucleate spores (Baker, 1944 ; Miller, 1946). The evidence, as it stands at present, therefore, by no means rules out a cytoplasmic origin of variation included under the heading of the " dual phenomenon ". Furthermore, every characteristic of the " dual phenomenon " can be paralleled by cytoplasmic determinants. The cultural, morphological and physiological differences which distinguish the components of a "dual " fungus can arise by cytoplasmic change. They arise spontaneously especially in aged material (Jinks, 1954, 1956, 1958, 1959 ; Gibson and Griffin, 1958 ; Sharpe, 1958 ; Marcou and Schecroun, 1959), they may be recovered from the wild and they are readily induced by a variety of treatments (Arlett, 1957 ; Jinks, 1958 ; Roper, 1958).

The changed cytoplasmic determinants are capable of existing side by side with their wild-type, unchanged counterparts (Pittenger, 1956 ; Arlett, 1957 ; Jinks, 1958, 1959 ; Gibson and Griffin, 1958 ; Marcou and Schecroun, 1959). These mixed cytoplasms or heteroplasmons can be produced in a manner analogous to heterokaryon formation (Gowdridge, 1956 ; Pittenger, 1956 ; Arlett, 1957 ; Gibson and Griffin, 1958 ; Jinks, 1959 ; Marcou and Schecroun, 1959) or may arise spontaneously by mutation in some, but not all, of the representatives of a cytoplasmic element within a spore or hypha (Arlett, 1957 ; Jinks, 1958, 1959).

Segregation from a cytoplasm of mixed origin has been obtained in five species of fungi (Pittenger, 1956 ; Arlett, 1957 ; Gibson and Griffin, 1958 ; Jinks, 1958, 1959 ; Marcou and Schecroun, 1959) and in others, observations compatible with such a segregation have been reported (Mahony and Wilkie, 1958 ; Roper, 1958 ; Sharpe, 1958). In fact, segregation from a cytoplasm of mixed origin has been used as a critical test for detecting functional differences between the components of the mixture (Arlett, 1957 ; Jinks, 1958, 1959).

Thus our present knowledge of the behaviour of mixed cytoplasms suggests that they could afford a satisfactory alternative to heterokaryosis as an explanation of the " dual phenomenon ".

With this possibility in mind four examples of the " dual phenomenon " in new isolates of *Penicillium* species of the *Assymetrica* group have been examined using our criteria for distinguishing between differences of nuclear and cytoplasmic origin in *imperfect* fungi. A cytological investigation showed that only one nucleus entered each asexual spore. Thus, irrespective of the final number of nuclei per spore they would remain homokaryotic. Hence a cytoplasmic basis of the " duality " was inferred for two of the four isolates when over 500 single spore propagations gave 17 per cent. " dual " colonies which were capable of further segregation. This was made even clearer when a mutation from green to white spores occurred in one component of one of these two isolates. This mutant segregated exactly as predicted for a nuclear controlled difference in a heterokaryon test with its wild-type partner, giving only white spored and green spored homokaryotic colonies. Furthermore, the spore colour difference segregated independently of the difference in colonial morphology and sporulation density which is the basis of the " duality " in this isolate.

Each of the two remaining isolates gave only two stable phenotypes in their asexual progenies ; one with normal sporulation and one which produced abundant mycelium but few spores. One of these isolates was investigated further. A white spore colour variant was induced in its normal sporulating component, and a heterokaryon made between this and its wild-type low sporing partner. In the asexual progeny of the heterokaryon, spore colour and sporulation density remained in their original association. Hence both the initial difference in sporulation density and the difference in spore pigmentation are presumably under nuclear control in this isolate.

Thus in our material the " dual phenomenon " may have either a nuclear or a cytoplasmic basis and hence a " dual " colony may be either a heterokaryon or a heteroplasmon. And while the importance of nuclear variation has long been appreciated, these results suggest that cytoplasmic variation is an equally important but neglected source of variation in wild *imperfect* fungi.

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REVIEWS

THE EVOLUTION OF DEVELOPMENT. By John Tryler Bonner. Cambridge: The University Press. 1958. Pp. 103+vii. 17s. 6d.

This pleasant little book, based on three lectures given at University College, London, looks at development in plants and animals in terms of evolution. Although this approach may not be as novel as the jacket advertisement would have one believe, it is a useful way of dealing with an important and difficult aspect of biology. In a hundred not very large pages of text, the author obviously cannot explore very far. He limits himself to a few organisms, one of them being the slime mould Dictostelium on which he has himself done much interesting and significant work.

Geneticists may have some difficulty in following Bonner's brief discussion of gene action. Consider, for example, the following sentences: "I do not mean to imply, however, that metabolism is run by genes, because except for synthesis it is not. The genes confine their activities to the manufacture of essential parts to keep the motor going; hence they are concerned with intracellular reproduction." It is not clear whether Bonner is referring solely to the primary role of genes in determining the structures of such macromolecules as ribonucleic acid and proteins, or whether he means also to include control of one or more steps beyond. From a later statement that ". . . one relatively simple aspect of gene action . . . that remains helpful and useful . . . is the idea that genes exert their effect primarily through the control of rates of processes," it would appear that he means the latter. If so, it is obvious that in this sense gene action is by no means limited to the control of synthesis. G. W. BEADLE.

BIOLOGICAL ASPECTS OF CANCER. By Julian Huxley. London: George Allen & Unwin. 1958. Pp. 156. 16s.

This is not a long book but it is an important one. Originating as a lecture delivered in New York, and developed as a review article which turned out to be considerably longer than expected, it presents the facts and problems of cancer as a biologist sees them. A wide variety of experimental evidence is surveyed, from plants as well as animals, the author using the term cancer to denote all malignant tumours. It is looked a from many points of view, cancer being, as is pointed out in the preface, a key subject for biology in its present state. There is a valuable list of some 500 references.

Seen genetically, perhaps the most important emphasis of the book is on cancer as the consequence of an escape of cells from the control and discipline of the genotype. Genetic unbalance, whether arising from wide crossing or inbreeding, expresses itself through upset of developmental processes which, if it involves abnormal persistence of cell proliferation, can lead to cancer. And once the cells have escaped from the normal genetic control, they take on an autonomy which endows them with a genetics of their own, so that they vary, are selected and evolve in their own right, within, of course, the limits of persistence imposed on them by the survival of their host or their transfer to new host individuals.

The genetical interest of cancer, and the importance of genetical principles for the study of cancer, thus emerge as beyond question. At the same time the genetical approach is but one of the many which are brought together in a treatment distinguished for the breadth of its view. Few experimental biologists can fail to derive stimulation from Huxley's presentation ; and indeed perhaps the best comment by way of summing up is to say that at least one reader is left with no doubt of the profit which, paraphrasing the words of the preface, biological science will derive from incorporating the cancer problem in its purview. KENNETH MATHER.

THE SPECIES PROBLEM. Edited by E. Mayr. Pub. No. 50 American Ass. Adv. of Science, Washington: Bailey Bros. and Swinfen Ltd., London. 1957. Pp. 395. 80s.

The classification of living organisms into species was developed originally for the convenience of identification. For this purpose the procedure for classifying was, and still is, a problem in itself. With the advent of Darwinism and, more recently, of genetics, it was natural to attempt to reconcile these units of identification with the units of variation and of evolution. Thus arose a new problem of a more profound and formidable character. A symposium to consider the problem was held at the meeting of the American Association for the Advancement of Science in Atlanta in 1955, the proceedings of which are recorded in this book.

The problem is defined and presented in an introduction by Professor Mayr. Contributions follow by Americans eminent in a wide variety of fields, genetics (H. L. Carson and T. M. Sonneborn), physiology (C. Ladd Prosser), embryology (J. A. Moore), palæontology (J. Imbrie) and systematics (V. Grant and J. L. Brooks). Professor Mayr sums up in the final paper.

There is, in general, agreement that the concept of the species in the modern, " biological ", sense is meaningful and worthwhile. On theoretical grounds the species concept has been enriched, and has benefited from, the infusion of genetic principles relating to gene flow and to genetic diversity as a basis for adaptive specialisation. In practice, as is made clear, there are difficulties of application, in particular to organisms reproducing asexually. The opinion of the majority of the contributors, and of Professor Mayr, that the benefits outweigh the difficulties, will not surprise and indeed will be endorsed by most biologists. Agreeable a feature though this may be it is perhaps not sufficient to encourage spending £4. There are, however, two other good reasons for recommending this book. Firstly, the papers are admirable, up-to-date summaries of results from an unusually wide range of investigations, each with an approach of its own, and each making its own special contribution to the same central problem. The result is thus to enlarge and extend one's appreciation of the concept of species and speciation. Secondly, Professor Sonneborn's article, dealing chiefly with *Paramecium*, is outstanding for its comprehensive analysis of natural populations considered from the standpoint both of individuals and of genetic systems. H. REES.

INSIDE THE LIVING CELL. By J. A. V. Butler. London: Allen & Unwin. 1959. Pp. 174, with 16 plates. 21s.

Professor Butler is a distinguished chemist who has worked on biological problems and in this book he discusses many of the topics which are important centres of biological research to-day. His subject matter ranges

from the structure and chemistry of cells to free will and the behaviour of the human brain. There are, among others, chapters on vitamins, hormones and antibodies ; genes, viruses and cancer ; photosynthesis, muscle and nerves ; tools, language and death. The book is written in a pleasant style, but there is no pretence at making difficult things simple. It is, of course, easy to quarrel with the presentation in such a book, but biologists who are not too learned will find it refreshing to read about transforming principles before conventional mutations : to have recombination in phage discussed without its jargon ; to have their frustration over pedigrees expressed so well.

The book is unfortunately marred by quite a lot of errors. Some of them ought to have been corrected in proof reading, but some are errors of fact. The most serious is perhaps the description of meiosis which gives a wrong impression of the mechanism. Another criticism which biologists will make is that natural selection is mentioned only in connection with ageing. Its significance for the other topics discussed is left out altogether.' Nevertheless this is a good book of a kind which we badly need, for it tells us what biologists are actually doing now and not what they were doing fifty years ago as so many books do.

F. A. L. Clowes.

SUBCELLULAR PARTICLES: Fifth Annual Symposium Publication of Society of General Physiologists. Teru Hayashi (Ed.). New York: Ronald Press Co. 1959. Pp. 213. $6.

Electron microscopy has made it possible to identify all the presumed types of genetic particle in the cell, to study their modes of development, possible relations and chemical activities. The eleven papers in this symposium open the new fields of enquiry ; they raise important questions and offer solutions of great genetic interest. Two of these are worth noting here.

The first refers to the question as to whether any given class of particles like the mitochondria, are physiologically versatile or genetically diverse. This question, opened by de Duve's discussion of lysosomes, will clearly dominate enquiry for some time to come. The second refers to the question as to whether one or several systems or channels exist through which RNA and DNA are propagated in the cell. Taylor and Wood's evidence of two systems of RNA (with slow and quick turnover) breaks new ground here.

Other valuable papers are by Novikoff, Green, Allfrey and Mirsky. Altogether the symposium is well designed. Summaries for all papers would have been useful : they serve both the author and the reader.

C. D. Darlington.

AN INTRODUCTION TO MEDICAL GENETICS. 2nd edition. By J. A. Fraser Roberts. Oxford University Press. 1959. Pp. 263. 35s.

BLOOD GROUPS (a Symposium). *Brit. Med. Bull.*, 15 (2). May 1959. Pp. 174. 20s.

SYMPOSIUM ON NUCLEAR SEX. Ed. by D. Robertson Smith and W. M. Davidson. Heinemann. 1958. Pp. 188. 21s.

The present three volumes demonstrate the range and character of the genetics which concerns, or may be thought to concern, those who teach and practice medicine. They also demonstrate the speed at which this range and character are changing.

Dr Fraser Roberts' work was designed, he explains, to show how genetic principles can be applied to medicine by *deductive* processes. The *Blood*

Group Bulletin and the *Symposium on Nuclear Sex* are designed to show how *inductive* processes can be used to discover new principles in genetics from the study of man and the practice of medicine. Thus the principles are being changed and the deductive processes may go awry. Yet in this second development (as shown in the *Bulletin*) Fraser Roberts is himself most profitably engaged. This is an embarrassing situation. How does it work out?

What happens is that in the *Introduction to Medical Genetics*, two simple and also artificial frameworks are fitted together. A large number of structural or physiological abnormalities are fitted to a corresponding number of "genes". These genes are defined on page 1 and classified by their dominant, recessive or intermediate, sex-linked autosomal "inheritance". They are fitted on the evidence of pedigrees and on the assumption that mankind is a freely inter-breeding population with a free exchanging of such Mendelian units. Only later do the complications appear. Only later is the biology of the situation introduced as a qualification of this perfect model. Even then all other organisms save man, and all other considerations save the strictly clinical and diagnostic are still excluded. And only scrappy references and a poor index are left to guide the novice out of the unreal situation his author has got him into.

Dr Fraser Roberts is not quite fair to his medical readers. He assumes that they will have only narrow technical interests and chiefly the narrow technical interests of twenty years ago. By making this assumption his *Introduction* deprives the subject of its intellectual possibilities. But has it not also deprived the subject of its practical possibilities? For surely the great practical lessons of genetics for medicine concern individuality and intelligence, fertility and survival, and specific resistance to infectious diseases, problems which do not submit to Mendelian analysis, problems which require reference to other animals, problems demanding integration as well as analysis, problems of the genotype rather than of genes. These are lessons which Fraser Roberts has admirably expounded elsewhere. It is only his framework that prevents him doing so here.

The two symposia are genuinely exciting works. The *Blood Group Bulletin* is almost entirely made up of condensed technical information. But it is so framed around its central theme of the blood group genes and their activities as to provide a rigorous statement of scientific methods in each technical connection, and in the whole system of connections, from the organic chemistry to the clinical treatment and from the linkage analysis to the evolutionary problem. There are about 1500 references.

The symposium on what is called "nuclear sex" is the fruit of the discovery ten years ago by Barr and Bertram in Canada that the resting nuclei of neurones in male and female cats were visibly different. Since then it has been shown that the female nuclei in many mammals show heterochromatin which is absent in the male. This has raised the question as to what happens in intersexes and also in sex-reversing, homosexual, and other doubtfully classifiable types. Twenty-two papers consider these and related problems. The results are usually inconclusive and sometimes unclear and disconnected. Never before have so many people met to discuss chromosomes who knew so little about them. For decency's sake their verbatim discussion might therefore have been omitted. The papers themselves, however, provide an introduction to a new world of—may we

say?—human genetics. It is the world of whole chromosomes, plus and minus, a world brought to life again at a point where *Drosophila* and various experimental plants went into cold storage twenty or thirty years ago.

Now, however, it is man and not a fly who is betraying his aberrations and our changed opinions are likely to reach beyond the boundaries of medicine or biology into new spheres of thought. This book therefore marks the beginning of an epoch. C. D. DARLINGTON.

TIERZÜCHTUNGSLEHRE. By W. Zorn *et al.* Stuttgart: E. Ulmer. 1958. Pp. 382. 134 figs. 28.80 DM.

This is a joint work of nineteen authors on the study of farm animal breeding and improvement. It covers the whole field from chromosome theory to artificial insemination technique, from the Watson-Crick model to the wild origins of domestic animals. Presumably it is intended as a text-book for university courses in agriculture rather than as a guide for practical breeders—the two requirements having wonderfully little in common. For either of them the conflict between classical genetics and practical policy will be evident to those who try to get to the root of the matter. At a superficial level, even, it is odd that the editor failed to notice the absurdity of assuming that whole chromosomes are units of recombination, male *Drosophila* with no crossing over being a pattern for all farm animals (p. 48). On this foundation the mathematical theory, although impressive, is top-heavy. C. D. DARLINGTON.

VAGANTEN, KOMÖDIANTEN, FIERANTEN UND BRIGANTEN. By Hermann Arnold. Stuttgart: Georg Thieme. 1959. Pp. 118, 30 figs. DM. 12.80.

SMOKE IN THE LANES. By Dominic Reeve. London: Constable. Pp. 180. 21s.

Side by side with the settled communities of developing agriculture, there have existed, at all stages of the evolution of society, unsettled communities following older ways of life. The wanderers have a poorer standard of living than the neighbours who have been displacing them and who have presumably arisen from them. They remain wanderers therefore, we are inclined to suppose, because they have lacked the skill to invent, the determination to carry on, or the power to control, the work of cultivation from which, as we think, an easier life comes. Here are two accounts of such people.

The first is an attempt, by studying the character and activities, the history, breeding and speech of all the different kinds of wanderers in a small part of Germany, the Palatinate, to understand why they behave in the way they do, and above all, how and why they maintain their independence and their integrity.

These groups vary between the extreme wildness of the gypsies and the semi-domesticity of certain trading and also thieving groups. They vary between the asocial, the anti-social or the parasitic character of pedlars, beggars, and solitary vagabonds, and the genuinely co-operative character of the travelling tinkers, comedians and circus folk. Yet all these groups stand apart from the settled people, the fixed and regular workers on the soil and in the city who are the indispensable and characteristic materials of civilisation.

How do they stand apart?

The author, who is a medical man, has been able to confirm the conclusions of earlier workers that mixture has slowly been taking place between some gypsy tribes and other vagrant and settled groups derived from the native European populations. This mixture partly arises from lasting sexual relations and partly from casual liaisons. It has been aided by the overlap in activities and interests between groups, but it is discouraged by their jealousy and competition.

These are the genetic foundations of the vagrant communities, according to Dr Arnold. Each group has its genetic character which maintains, and is maintained by, its individual mode of life. But some migration, some gene-flow—and some word-flow also—takes place between the groups. And this flow is greater in some cases than in others. The gypsies of the remoter Palatinate, for example, were—but no longer are—purer than those of Swabia.

There are probably no pure gypsies in Europe to-day. From time to time they have bred with "white" wanderers who abounded in Europe before they came. But those gypsies who claim to be pure "Romany" do so because they have kept their language and their customs. A larger progeny of cross-breeding has at the same time passed over to the other wandering groups, the "Jenischen" in Germany, the "Didikai" in England, the "Tattare" in Sweden, and so on. These groups arose from race-crossing largely during the gypsy persecutions between 1539 and 1800. They came into the administrative records shortly before 1800, chiefly on account of their conflicts with the law. Each group of wanderers has its own territory. It constitutes a geographical race, and like all such human races, it has its own dialect.

The groups to which these names of Jenischen, Didikai and so on, are applied are in some respects of genuinely lower culture and intelligence than the gypsies. They are less well adapted than the gypsies to a life so little supported by labour. The gypsies learnt how and when to catch hedgehogs. The cross-breeds still know this but they have forgotten when *not* to catch hedgehogs: they suffer less restraint; they show less foresight. The cross-breeds moreover sometimes settle down. Sometimes it is for peddling, sometimes for collecting rags and old iron, sometimes it leads to crime—chiefly petty theft, rarely grand robbery. They change their habits and occupations more readily than the gypsies. They change when it pays them to change. The pedlars of Germany were registered to the number of a quarter of a million in 1896, when an alteration of the law favoured this status. At another time they may seem to be assimilated in the welfare state. A large body of apparently settled people are willing to assume an unsettled life, but they are people who can be shown to be derived from crossing with the vagrant population. Such groups in Germany are even more numerous and diverse than the costermongers, barrow boys, hop-pickers, knife-grinders and barrel-organ men whom we meet in other European countries—or in the pages of Mayhew.

Certain of these groups reveal a sex differentiation in their relation with crime which they derive, like so much else, from their gypsy ancestry. In these groups, only women are found to be overtaken by the law. Why? The wife does all the handiwork and also sells it. The proceeds she has to give to her man—her bully or ponce, as we might call him in city life.

If she does not give him enough he will beat her. So she steals. And he keeps within the law. This, Dr Arnold shows, is how the society works.

The consequences of cross-breeding of settled and wandering stocks is exactly revealed by Dr Arnold's enquiries. It is to produce families which are mixed in their dispositions, the disposition showing itself definitively by the marriage of sibs into the opposed groups. Some pass over by marriage from wanderers to peasants (becoming *verbauert*) or *vice versa*, the others remaining as they were brought up. Sometimes a man may make several unions inside and outside the circus community, and their progenies, all brought up in the same caravan, show the different genetic effects of their different mothers. We may doubt whether Dr Arnold is right in attributing this result to a single gene difference. But when a part of the children break with the life and with the family they have been brought up in, he is certainly right in attributing it to genetic recombination followed by a genetic determination of behaviour.

The movement of gypsy descendants into irreversibly settled life is slight owing to their largely cross-breeding with unsettled whites. One instance, however, Arnold describes of a village, Neumühle, where gypsy infusion can be shown a hundred years ago. He is able to trace the influence on the character and intelligence, habits and speech of the people even after they have later migrated elsewhere. The same kind of slum life with a copious crime record has been produced that we meet here and there in other parts of Europe and America often, although not always demonstrably, for the same kind of reason.

A part of the cross-bred population thus suffer some disadvantage from comparison with the pure gypsies. They have lost the art, the music, the elegance and some of the wisdom of the pure race. They are detribalised as a result of crossing with the simplest or the lowest in the " white " communities. Smaller groups, however, have fared otherwise. They have bettered their cultural and economic status. They have done so, as Arnold shows, by entering into, and by helping to build, a more complicated social structure. These are the entertainers, a breed which has become many breeds or societies, those based on the fair, the circus, and the race-course. Here we find the animal trainers, the showmen, the clowns, the acrobats, the swing and merry-go-round men, the Punch and Judy men, the puppet players, knife-throwers and jugglers.

The genealogy of these circus people can be traced back to Magdeburg in 1417. The strong men and tight rope walkers of early days have been successively reinforced—culturally and genetically by new people and new talents. The pedigrees and the records of two centuries enable Arnold to show how this has happened. First there were the Spanish trick riders by way of the Vienna Prater ; then the French " cirque equestre " a century later ; these were followed by a variety of other entertainers including nigger minstrels. From these have further diversified the entertainers of the world-wide twentieth century circus. And as they have diversified, they have, like all such societies, become stratified. The gypsy-mixture is most obvious in the lower strata, while the upper strata where initiative, enterprise and wealth are concentrated may be free from gypsy or didikai ancestry. The whole system forms a hierarchy of genetically varied and socially co-operating skills and abilities.

The evidence of language is not alone decisive in tracing the origins of vagrant populations. But it is a valuable indication. " Rotwelsch ", which is known to us as " thieves' cant " since the work of Bampfylde Moore Carew, is a term Dr Arnold uses to describe the collective forms of speech of the vagrant populations in Germany. These he traces back to, and compares with, its classical source in the *Liber Vagatorum* of 1510.

In the *Liber* were ten per cent. of Hebrew words. After the Thirty Years' War, Romany began to enter into the mixed vocabulary and during the eighteenth century there was a further large influx of Yiddish. The Romany component came with cross-breeding ; the Hebrew however, according to Church records, did not seem to do so. It is only in the robber-bands which flourished in Napoleonic times in the Rhineland that Jewish mixture is evident.

The results of this evolution Arnold illustrates by showing the etymological origins of 500 words in present usage among seven types of vagrant group. The speech of each trade-group is distinguished, so Arnold argues, in each locality from that of other trade-groups. And all of them are distinct from urban forms of gutter-speech which have no trade character and consequently less stability and less historical value.

Thus, just as the Romany dialects have picked up fragments of European languages while the gypsies have been moving west, so the vagrant jargon has clearly picked up words from Romany and Yiddish as well as the native European languages, and in different proportions according to the social and genetic connections of each group.

The study of vagrants in modern Europe is only a small part of the study of wandering peoples throughout the world. And, since it shows the genetic origin of the criminal and the parasite, it is one of the less edifying parts. We might also reasonably derive our aristocracies, our fighting castes, our tradesmen, craftsmen and artists from hunting and pastoral ancestors who have been pushed out of the richer lands of the earth by sedentary cultivators, and have cross-bred with them. What is important in Arnold's study is in his showing us a method of attack on the genetics of society. His records and his pedigrees, his historical and linguistic studies offer us a model of how the effects of mixing races may be revealed in the process of sorting out. The contrast between the nomadic and settled types of life is combined with, and marked by, the physical contrast between Romany and Gorgio and the cultural contrast between their languages and their beliefs. The communities concerned are small, distinct and well recorded. Their evolution has consequently been rapid. Everything has conduced to elucidate the genetic processes in the mixture and recombination of the two kinds of people. Recombination gives diversity of genotype, which may create many new cultures ; or may destroy one old culture. Are not these processes characteristic of the evolution of all human societies ? There seems no reason to doubt it.

This book is well illustrated. But a quarter of its reading matter is cut up into footnotes which are crowded at the end. This is an old tradition of learned works But this learned work deserved to be better presented.

The second book, complementary to the first, is an autobiographical sketch by Dominic Reeve. Here we see life and society from the point of view of a first-generation cross, a Didikai, who lives as a traveller though married to a Gorgio (English) wife. His story is authentic in its Hogarthian

detail and it is told with grace and distinction. The author recognises, as Arnold does, the importance of Romany admixture in various house-dwelling groups from the poor ragmen to the rich dealers. He disclaims any knowledge of genetics. But he says a great deal about the official and unofficial racial discrimination (as it is now called) in England against gypsies. He discusses the causes and the consequences of inbreeding and outbreeding in the communities he knows. And everything he says has a lesson for students of the genetics and evolution of society.

C. D. DARLINGTON.

SOCIAL THEORIES OF FERTILITY AND THE MALTHUSIAN DEBATE. By D. E. C. Eversley. Oxford: Clarendon Press. 1959. Pp. 313. 35s.

Malthus' writings, like those of Charles Darwin, are remarkable for their inconsistencies and ambiguities. They have the scriptural character of being quotable for contrary purposes. The present work does not, unfortunately, do much to remove the confusion that Malthus created. This is due to Dr Eversley's having reached no very definite opinions of his own, a failure for which his premises and his method are responsible.

Dr Eversley's account deals with discussions of human population and fertility throughout history. He is concerned with what people said and who influenced whom. It is indeed in the manner of the political or literary historians that the author's mind works, and it is their sources that he uses.

Take the problem of instincts or appetites discussed by Malthus. It is, one might think, in its foundations a biological problem. Malthus decided that food came first, and sex second. The possibility that instincts or appetites were genetically determined, subject to heredity, and enormously variable as between individuals, some of whom are not troubled by sexual appetites, did not occur to Malthus. It did not occur to any of his successors so far as the present author has discovered. And it does not occur to the present author himself. He has not noticed what Erasmus Darwin or Francis Galton said on the related problem of the infertility of heiresses. He has read what Sir William Temple and H. Spencer said on the matter. But it happens that Darwin and Galton were right while Temple and Spencer, these confident bachelors, were wrong. That, to a biologist, matters a great deal and perhaps historians will one day think the same.

Dr Eversley's subject is so important and his work so painstaking that it cannot be without value. But it is a pity he did not think out its foundations before he began, or discuss the matter with someone who had thought them out.

C. D. DARLINGTON.

AN OUTLINE OF HUMAN RELATIONSHIPS. By Eustace Chesser (with a Preface by Sir Cyril Burt). London: Heinemann. 1959. Pp. 446. 25s.

Here Dr Chesser, in his nineteenth volume, sums up his views on psychology, life and love. What he says has been said before, but rarely at such length. Dr Chesser asks how many of our ideas are original (p. 352). Well, indeed, may he ask. But often they wear a new dress. There are, he says, two kinds of heredity : *genetic heredity* and *conditioned heredity* (p. 123). This is a variation on the theme of " social heredity " and all such variations are, we must admit, genuine contributions to muddling the

layman. So also is the statement that the environment acts like heredity "for all practical purposes" (p. 75). Conclusions follow in keeping with these premises. The sixteen-page preface, although more instructive than the book, is not at all inappropriate. C. D. DARLINGTON.

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